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STUDIES ON PLANT GROWTH-REGULATING SUBSTANCES. V. STERIC FACTORS IN RELATION TO MODE OF ACTION OF CERTAIN ARYLOXYALKYL-CARBOXYLIC ACIDS*

By M. S. SMITH, R. L. WAIN AND F. WIGHTMAN

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(With Plate 12 and 4 Text-figures)

Three synthetic growth-regulating substances, α -(2-naphthoxy)-, α -(2:4-dichlorophenoxy)- and α -(2:4:5-trichlorophenoxy)-propionic acids, have been resolved into their (+)- and (-)-forms. The physiological activity of these isomers and of the racemic compounds has been investigated using the pea curvature and *Avena* cylinder test methods of assessment.

The (+)-isomers in each case displayed high activity in both tests, whereas that exhibited by the (-)-isomers was negligible. The racemic compounds showed intermediate activity.

The mode of action of aryloxyalkylcarboxylic acids is discussed in the light of the results presented. These considerations suggest the possibility that the activity of the (+)-isomers might be antagonized by the presence of the corresponding inactive enantiomorphs. Our experiments show that such antagonism does in fact operate.

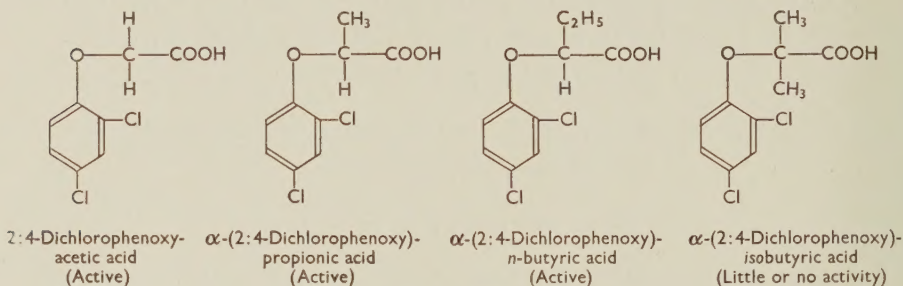
INTRODUCTION

Plant growth-regulating activity can be shown by a variety of organic substances, such as carbon monoxide, ethylene and certain benzoic, aryl and aryloxy acids. Though such substances, of widely diverse chemical structure, may often induce a similar biological response, it would seem improbable that a common mode of action is involved. If we accept the possibility that a growth response may arise from an interference at different points in the complex series of biochemical reactions associated with growth, then the means by which such interference is achieved may differ with compounds of varying structure, though the final growth response may be similar. On this basis, this initial interference may be regarded as the first stage in the mode of action of such compounds.

Of the synthetic growth-regulating substances now available, the aryloxyalkylcarboxylic acids form the most important group. In this series, high growth-regulating activity is often exhibited by the acetic, α -substituted propionic and *n*-butyric acid derivatives, all compounds in which the carbon atom adjacent to

* The investigations described in this paper were presented by one of us (R.L.W.) in a lecture delivered at the XIIth International Congress of Pure and Applied Chemistry, New York, September 1951.

the carboxyl group carries at least one hydrogen atom. In the α -substituted *iso*-butyric acids, where this requirement is not fulfilled, negligible activity is shown (Osborne & Wain, 1951 *a*, *b*).



Since the α -aryloxypropionic acids possess an asymmetric carbon atom, they exist in *dextro* and *laevo* rotatory forms. It has recently been shown (Smith & Wain, 1951) using a range of biological tests, that the (+)-isomer of α -(2-naphthoxy)propionic acid is a highly active growth substance, whereas the (-)-isomer possesses little or no activity. Although this was the first comprehensive study of stereoisomers in the aryloxy series, Kögl & Verkaaik (1944) had previously examined the growth-regulating activity of the enantiomorphs of α -(indole-3)propionic acid. In contrast to our results with α -(2-naphthoxy)propionic acid, they observed that different relative activities were exhibited by the two isomers when examined by two test methods. Thimann (1951) has stated that in the pea curvature test the (+)-isomer of α -(2:4-dichlorophenoxy)propionic acid has approximately twice the activity of the racemic form. Veldstra (1951), also using this test, has shown that (-)-1:2:3:4-tetrahydro-1-naphthoic acid is highly active, whereas the (+)-isomer has little activity.

In our previous paper (Smith & Wain, 1951), suggestions on the mode of action of aryloxy acids were put forward. It was shown that the results obtained could be explained by a mechanism involving either (*a*) combination of the active growth substance with an optically active cell constituent, or (*b*) the association of the unsaturated ring system, the carboxyl group and the α -hydrogen atom with specific receptor groups at the site of action. This work has now been extended; α -(2:4-dichlorophenoxy)propionic and α -(2:4:5-trichlorophenoxy)propionic acids have been resolved and the growth-regulating activity of their stereoisomers examined. The results are here presented and discussed in the light of the above hypotheses. Further, it has been shown that the inactive enantiomorph in each case can, under certain conditions, antagonize the activity of the corresponding active isomer. The theoretical implications of this finding are discussed.

CHEMICAL SECTION

Preparation of α -(2-naphthoxy)propionic acid

4.6 g. sodium (0.2 g. atom) was reacted with excess ethanol and 28.8 g. of recrystallized 2-naphthol (0.2 mole) added. To the clear solution obtained on warming, 36.2 g. of ethyl α -bromopropionate (0.2 mole) was added and the mixture was boiled under reflux for 4 hr. 200 ml. 10% NaOH was added, and heating was continued for a further hour to hydrolyse the ester. The alcohol was distilled off and the acid was precipitated with excess ice-cold hydrochloric acid. Yield 32 g. (74%). The acid was recrystallized twice from water and once from light petroleum giving white needles, m.p. 107–108.5° C.

Resolution

This was carried out by fractional crystallization of the (+)-cinchonine salts as described in our earlier paper (Smith & Wain, 1951), in which the specific rotations are given.

Preparation of α -(2:4:5-trichlorophenoxy)propionic acid

This was carried out exactly as described above, employing 2:4:5-trichlorophenol in place of 2-naphthol, and refluxing for 10 hr. Yield 48 g. (89%). Recrystallized twice from 25% ethanol to give white needles, m.p. 179–180.5° C.

Resolution

This was achieved in two stages by forming (1) the salt of the (–)-acid with (–)-strychnine, and (2) the salt of the (+)-acid with (+)-yohimbine. In each case one equivalent of the racemic acid was treated with half an equivalent of the optically active base and half an equivalent of sodium hydroxide.

Preparation of salt of (–)-acid with (–)-strychnine

16.7 g. of finely powdered strychnine (m.p. 273–275.5° C.) was suspended in 2 l. boiling 20% ethanol. To the vigorously stirred suspension, 27 g. α -(2:4:5-trichlorophenoxy)propionic acid in 50 ml. N-NaOH and 50 ml. ethanol was added. The mixture was brought to boiling with continued stirring. The almost clear solution was filtered and, on cooling, the salt separated. Yield 26.9 g. It was recrystallized five times from 20% ethanol. Yield 7.8 g.

Preparation of salt of (+)-acid with (+)-yohimbine

19.5 g. of yohimbine hydrochloride was dissolved in 1 l. boiling water. The base was precipitated with excess ammonium hydroxide, filtered and washed with hot water. The moist base was suspended in 2 l. 25% ethanol, and a mixture of 27 g. α -(2:4:5-trichlorophenoxy)propionic acid in 50 ml. N-NaOH and 50 ml. ethanol added to the vigorously stirred suspension. The solution cleared completely, and

in a few minutes the salt began to crystallize as a mass of very fine needles. Yield 28 g. Recrystallized three times from acetone. Yield 9.4 g.

Regeneration of acids

Each salt was treated with a slight excess of sodium hydroxide and the base removed by filtration. The acids were recovered by addition to an excess of ice-cold dilute hydrochloric acid and recrystallized from 30% ethanol.

Yield of (–)-acid 3 g. (from 7.8 g. strychnine salt).

Yield of (+)-acid 3.3 g. (from 9.4 g. yohimbine salt).

Specific rotations were as follows:

(–)-Acid (m.p. 143–144.5° C.). 550.1 mg. in 25 ml. ethanol; 2 dm.; $\alpha = -2.19^\circ$; $[\alpha]_D^{20.5} = -49.7^\circ$.

(+)-Acid (m.p. 143–144.5° C.). 589.9 mg. in 25 ml. ethanol; 2 dm.; $\alpha = +2.31^\circ$; $[\alpha]_D^{22} = +48.9^\circ$.

α -(2:4-Dichlorophenoxy)propionic acid (m.p. 117° C.). This was prepared and resolved by Mr F. E. Smith of Pal Chemicals, Ltd. The (+)-acid was isolated through its (–)-brucine salt; the (–)-acid through its (+)-cinchonine salt.

(+)-Acid. Colourless needles from benzene (m.p. 124° C.). $[\alpha]_D^{21} = +26.6^\circ$ (C, 1.23) in alcohol.

(–)-Acid. Colourless needles from benzene (m.p. 124° C.). $[\alpha]_D^{20} = -26.3^\circ$ (C, 1.22) in alcohol.

BIOLOGICAL SECTION

Assessment of growth-regulating activity

Two standard laboratory methods of assessment were employed, viz. the pea curvature test and the *Avena* cylinder test. Experimental techniques were modified to obtain material showing maximum sensitivity and consistency in response to growth substance treatment.

(1) *The pea curvature test* (Went, 1934).

Segments (5 cm.) are removed from young internodes of pea stems and split longitudinally through the elongating region. The split halves develop an outward curvature due to the tension normally existing in the epidermal cells. When such segments are placed in a solution of a growth substance, a differential rate of extension growth occurs in the inner and outer tissues resulting in the split halves developing an inward curvature (Van Overbeek & Went, 1937). The final state of the curvature is usually reached after some 18 hr. at 26° C., and has been shown by Went (1934) to be dependent on the concentration of the compound employed. In water controls no growth response is observed and the split halves retain their original outward curvature. In assessing the growth response, a scale similar to that suggested by Went (1934) was employed. Details of experimental procedure are to be found elsewhere (Went & Thimann, 1937; Kent & Gortner, 1951).

(2) *The Avena cylinder test.*

This depends on the linear growth response shown by short cylindrical sections of *Avena* coleoptile when treated with solutions of growth substances (Bonner, 1933).

Bentley (1950) critically examined the variable factors in the test and recommended a revised experimental procedure which was adopted in the present investigation with some further modifications in technique. Uniform coleoptiles, 2 cm. in length, are used as experimental material. With the aid of a specially designed brass cutting instrument (Pl. 12, fig. 1), 10 mm. sections are rapidly and accurately excised from a batch of ten coleoptiles; at the same time, apical 3 mm. segments are removed. The latter, since they contain much of the natural auxin, are rejected. The 10 mm. sections (ten for each test) are threaded on sealed glass capillaries and floated on the test solution. After 24 hr. at 26° C. (Pl. 12, fig. 2), the sections are measured to the nearest 0.5 mm. under the microscope. Distilled water controls are included in each experiment, and in addition the response of sections placed in a 1 p.p.m. solution of 2:4-dichlorophenoxyacetic acid (sodium salt) is determined. Results are calculated as the excess elongation of the test sections over the final length of the water controls, and are expressed as a percentage of this length.

All compounds in the present investigation were examined at the stated concentration of the acid in the form of its sodium salt.

EXPERIMENTAL RESULTS

(a) *The growth-regulating activity of the racemic and optically active compounds*

The activities of ($\pm$)-, (+)- and (-)- α -(2-naphthoxy)-, α -(2:4-dichlorophenoxy)- and α -(2:4:5-trichlorophenoxy)-propionic acids in the *Avena* cylinder test are assembled in Table 1. Curvatures obtained in the pea test are shown photographically for the 2:4-dichloro- and 2:4:5-trichloro-compounds in Pl. 12, figs. 3 and 4. The corresponding results for the 2-naphthoxy acid have already been published (Smith & Wain, 1951).

(b) *Influence of one stereoisomer on the activity of its enantiomorph*

The effect of increasing concentrations of the (-)-isomers of the three propionic acids on the activity of the corresponding (+)-isomer has been examined in the *Avena* cylinder test. The results are shown graphically in Text-figs. 1-3.

DISCUSSION

The present investigation has been concerned with the growth-regulating activity of the (+)-, (-)- and racemic forms of α -(2-naphthoxy)-, α -(2:4-dichlorophenoxy)- and α -(2:4:5-trichlorophenoxy)-propionic acids. Though the absolute configuration

of the stereoisomers has not been established, it is known that $(-)\text{-}\alpha\text{-bromopropionic}$ acid is the L-form (Brewster, Hughes, Ingold & Rao, 1950). Further, it is well known that the reaction between naphtholate and bromopropionate ions (S_N2 type) should lead to a simultaneous inversion of the configuration. Thus the $(+)\text{-}\alpha\text{-}(2\text{-naphthoxy})\text{propionic}$ acid, itself an active growth substance, is considered to possess the D-configuration (Fredga & Matell, 1951). Since the other two $(+)\text{-}$ isomers studied in this investigation are also active growth-regulating substances it is probable that they too have the D-configuration.

TABLE 1. *Activity of $(+)\text{-}$, $(\pm)\text{-}$ and $(-)\text{-}$ forms of $\alpha\text{-}(2\text{-naphthoxy})\text{-}$, $\alpha\text{-}(2:4\text{-dichlorophenoxy})\text{-}$ and $\alpha\text{-}(2:4:5\text{-trichlorophenoxy})\text{-propionic}$ acids in the *Avena* cylinder test*

α -(2-Naphthoxy)propionic acid (concentrations in p.p.m.)							
Treatment	0.5	1	5	20	50		
Water controls	100	100	100	100	100		
(+)-Acid	101	103	108***	118***	127***		
($\pm$)-Acid	99	100	101	105*	106**		
(-)-Acid	100	100	101	99	97		
α -(2:4-Dichlorophenoxy)propionic acid (concentrations in p.p.m.)							
Treatment	0.05	0.1	0.5	1	5	20	50
Water controls	100	100	100	100	100	100	100
(+)-Acid	114***	125***	143***	152***	142***	138***	135***
($\pm$)-Acid	104	110**	122***	137***	145***	138***	137***
(-)-Acid	101	100	99	97	97	98	98
α -(2:4:5-Trichlorophenoxy)propionic acid (concentrations in p.p.m.)							
Treatment	0.01	0.05	0.1	0.5	1	5	20
Water controls	100	100	100	100	100	100	100
(+)-Acid	124***	145***	148***	152***	153***	155***	146***
($\pm$)-Acid	108*	129***	138***	148***	151***	152***	142***
(-)-Acid†	100	99	98	99	97	97	97

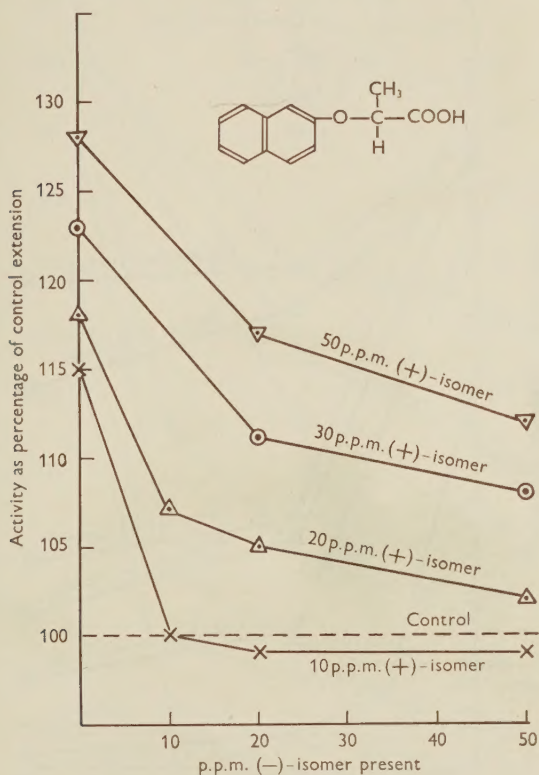
Results significantly different from control: *, at 5% level; **, at 1% level; ***, at 0.1% level.

† In a separate test, activity of $(-)\text{-acid}$ at 50 p.p.m. = 103.

The results obtained in both the pea curvature and *Avena* cylinder tests for the racemic and optically active forms of these three propionic acids, reveal in each case a high degree of growth-promoting activity in the $(+)\text{-}$ isomer, almost complete absence of activity in the $(-)\text{-}$ isomer, with the racemic form showing an intermediate activity (Table 1; Pl. 12, figs. 3 and 4). These results are in complete agreement with those previously reported from this laboratory for the three forms of the naphthoxy acid, using a wide range of biological tests (Smith & Wain, 1951). It should be noted that the present investigation includes the results of a re-examination of the racemic and optically active forms of $\alpha\text{-}(2\text{-naphthoxy})\text{propionic}$

acid by the *Avena* cylinder test. These results are closely similar to those reported in our previous paper (Smith & Wain, 1951).

In animal physiology there are numerous instances where the two stereoisomeric forms of a substance differ widely in their biological effect (e.g. see Cushny, 1926). Some experiments on the behaviour of stereoisomers in inducing growth responses

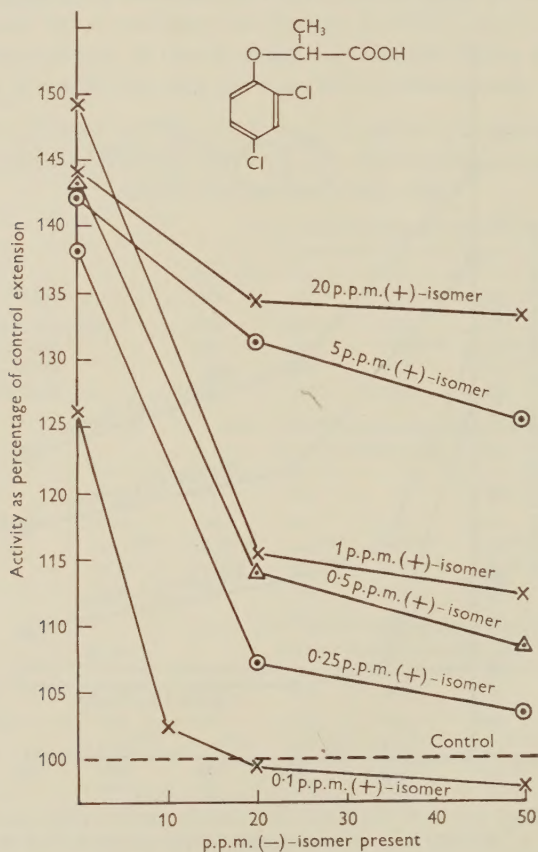


Text-fig. 1. Activity of (+)- α -(2-naphthoxy)propionic acid in presence of increasing amounts of the (-)-isomer in the *Avena* cylinder test.

in plant tissue have been reported by Kögl & Verkaar (1944), Veldstra (1951), and by Thimann (1951), and we have demonstrated that the stereoisomeric forms of a growth-regulating substance can not only differ widely in physiological activity, but can show the same relative activity in inducing a wide range of growth responses in which both excised plant tissue and intact plants are involved (Smith & Wain, 1951).

Studies on growth-regulating activity in relation to chemical structure have been made by a number of workers (for review see, for example, Wain, 1949; Audus,

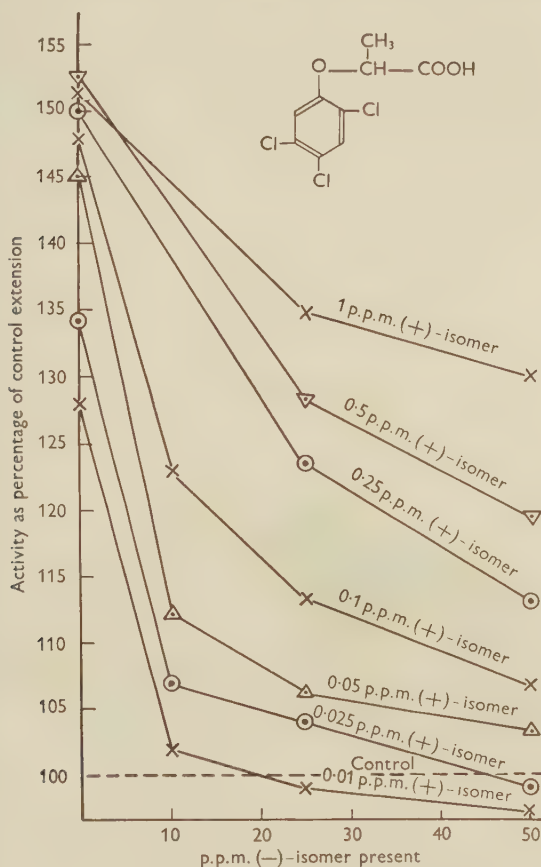
1949; Thimann, 1951). Most of our work in this field has been concerned with the aryloxyalkylcarboxylic acids and their mode of action (Wain, 1951). There is considerable evidence to show that in this type of compound an unsaturated ring system and a carboxyl group are necessary for activity (Koepfli, Thimann &



Text-fig. 2. Activity of (+)-α-(2:4-dichlorophenoxy)propionic acid in presence of increasing amounts of the (-)-isomer in the *Avena* cylinder test.

Went, 1938; Veldstra, 1944*a, b*), and, in addition, Osborne & Wain (1951*a*) have indicated that at least one hydrogen atom must be attached to the α-carbon atom (i.e. the carbon atom adjacent to the carboxyl group) (see formulae on p. 296). One of the primary requirements in growth regulation by means of chemicals is that the molecule of the active compound must reach the site of action within the tissue, a property itself largely dependent on molecular structure and possibly related to the balance between the lipophilic and hydrophilic parts of the molecule (Veldstra &

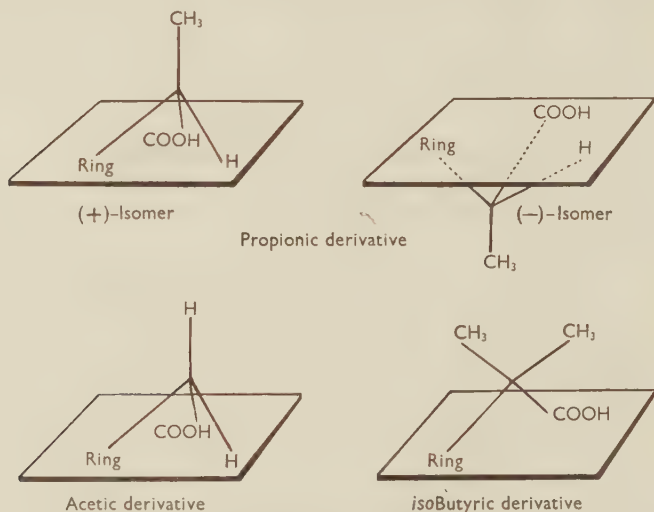
Booij, 1949). Having reached the site of action, there still remains the question of the possible function of the specific groupings in the molecule in initiating the growth response. As stated above, an unsaturated ring system, carboxyl group and an α -hydrogen atom appear to be necessary. These three groupings are attached



Text-fig. 3. Activity of (+)- α -(2:4:5-trichlorophenoxy)propionic acid in presence of increasing amounts of the (-)-isomer in the *Avena* cylinder test.

to the asymmetric carbon atom in α -aryloxypropionic acids, such as the three investigated in the present work. If we assume that these essential groupings become associated with three specific receptor centres in a cellular boundary constituting the site of action, then it is clear that only one of the stereoisomers can present these three groupings in the correct relative positions. Its enantiomorph can only do so if the fourth grouping actually penetrates the boundary (see Text-fig. 4).

Such a mechanism could explain our experimental results not only with stereoisomers of aryloxypropionic acids, but also with the aryloxy acids in general. Thus the acetic acid analogues of all the three propionic acids used in this investigation are highly active growth substances, whereas the corresponding *iso*-butyric acids have only slight activity (Osborne & Wain, 1951*a*). Such relative activity is precisely what would be expected on the basis of the above hypothesis, for the acetic acid molecules, irrespective of their orientation, are capable of presenting the three essential groupings to the appropriate receptor centres. On the other hand, the *iso*-butyric acid molecules can only provide two of the requisite groupings (see Text-fig. 4), so with these compounds activity will depend on the



Text-fig. 4. Diagram illustrating the presentation of essential groupings to receptor centres.

degree to which the third grouping is necessary. Since all the aryloxy*iso*butyric acids so far studied have little or no activity, the methyl group presumably has no specific function. It should be noted that these considerations can apply only if the growth-substance molecule does not undergo chemical modification or racemization within the plant tissue to yield a molecule possessing different growth-regulating activity.

If we accept the above hypothesis on mode of action, it is evident that the receptor centres at the site of action play a vital role in the growth-regulating activity of aryloxy acids. Further, any inactive compound which could provide groups capable of engaging with these receptor centres might, by a 'blocking' effect, prevent an active compound from inducing a growth response. Such considerations led us to examine, in the *Avena* cylinder test, the activity of

(+)- α -(2-naphthoxy)-, (+)- α -(2:4-dichlorophenoxy)- and (+)- α -(2:4:5-trichlorophenoxy)-propionic acids in presence of increasing amounts of their corresponding inactive enantiomorphs. The results (Text-figs. 1-3) show conclusively that the inactive (-)-isomer can reduce, and even eliminate, the activity of the (+)-isomer in this test.

The results examined in detail are of interest, for if we assume that both the (+)- and (-)-stereoisomers are equally capable of providing groupings to associate with the receptor centres, then in mixtures of both forms, the degree to which activity will be shown must depend on the relative amounts of each present. Thus, for example, in Text-fig. 3, the activity of 0.01 p.p.m. of the (+)-isomer of α -(2:4:5-trichlorophenoxy)propionic acid is neutralized by 10 p.p.m. of the corresponding (-)-isomer (i.e. 1/1000 ratio), whereas the activity of the (+)-isomer at 1 p.p.m. is only partially neutralized by 50 p.p.m. of the (-)-isomer (i.e. 1/50 ratio). With higher concentrations of the (+)-isomer it was not possible to study the effect of the (-)-isomer in large excess because of the complication of phytotoxicity. All the present results, then, are consistent with the idea that molecules of the inactive stereoisomer can block the receptor centres required by the active enantiomorph to initiate a growth response in the *Avena* cylinder test. Although the present results have been obtained with solutions of the sodium salts, it might be mentioned that in separate experiments with (+)- and (-)- α -(2:4:5-trichlorophenoxy)propionic acid as free acids, we have again clearly demonstrated that antagonism by the inactive isomer operates.

There remains, however, an alternative explanation of all results discussed above. To explain the specificity in physiological action often shown by (+)- and (-)-isomers of a compound in the animal body, it has been suggested that combination occurs with some optically active cell constituent. This reaction leads to the formation of two compounds with different properties and it is assumed that only one of these, that formed from the active isomer, can bring about the biological response (e.g. see Cushny, 1926). Not only does this theory equally well explain our results with the separate stereoisomers but it also accounts for the antagonistic effects of the (-)-isomers which we have demonstrated. Thus the (+)-isomer would not be expected to compete favourably with a large excess of the (-)-isomer for reaction with the optically active cell constituent.

It would therefore appear that both hypotheses discussed above could satisfactorily explain our results, though the necessity for the presence of unsaturated ring, α -hydrogen atom and carboxyl group, is perhaps best accounted for by the mechanism involving receptor centres.

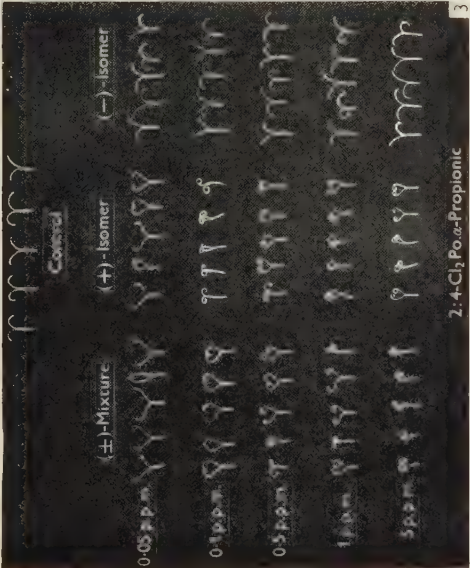
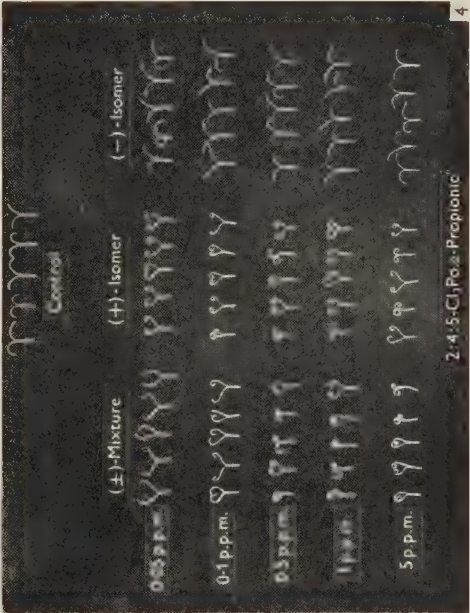
Returning to this first hypothesis, it has been indicated that the inactive (-)-isomers might exert their antagonistic effect by blocking all three receptor centres with which the three essential groupings of the active (+)-isomers seek to engage. However, as already pointed out, this possibility could only arise if the inactive molecule penetrated the surface boundary. It is possible, however, that the

observed reduction in growth response could arise by the (–)-isomers blocking at two types of receptor centre only. Though such considerations are beyond the scope of the present discussion, the question does arise whether other inactive substances which can provide only two of the essential groupings (e.g. the corresponding aryloxyisobutyric acids), or even only one such group (e.g. the corresponding phenol or naphthol), are capable, by a similar mechanism, of antagonizing the growth-regulating activity of aryloxy acids in general. Investigations along these lines are now in progress and the results will be presented in a subsequent paper.

The assistance of Mrs E. Lawson in the biological testing and of Mr D. Simons for photographs is gratefully acknowledged. Thanks are also due to Mr F. E. Smith for providing the specimens of α -(2:4-dichlorophenoxy)propionic acid. The work was made possible by the award to one of us (F.W.) of a College Fellowship provided by Messrs May and Baker Ltd.

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EXPLANATION OF PLATE 12

- Fig. 1. Cutting instrument used in *Avena* cylinder test.
- Fig. 2. Growth responses shown in *Avena* cylinder test.
- Fig. 3. Activity of ($\pm$)-, (+)- and (-)-forms of α -(2:4-dichlorophenoxy)propionic acid in the pea curvature test.
- Fig. 4. Activity of ($\pm$)-, (+)- and (-)-forms of α -(2:4:5-trichlorophenoxy)propionic acid in the pea curvature test.

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THE PHYTOTOXIC PROPERTIES OF ALTERNARIC ACID IN
RELATION TO THE ETIOLOGY OF PLANT DISEASES
CAUSED BY *ALTERNARIA SOLANI* (ELL. & MART.)
JONES & GROUT

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(With Plate 13 and 2 Text-figures)

Alternaric acid, a metabolic product of some strains of *Alternaria solani*, is highly phytotoxic. If introduced into the vascular system of cut shoots of tomato or potato, it travels upwards and causes necrotic lesions of the stem, petioles and leaf blades, very similar in appearance to the lesions appearing in some phases of natural attack by *A. solani*. Alternaric acid also produces similar lesions on plants outside the host-range of *A. solani*. Associated with the production of visible lesions is a disturbance of the water-balance of shoots. Transpiration is increased and, in spite of a simultaneous but smaller increase in water uptake, this eventually leads to complete desiccation of the shoots. A strain of *A. solani* known to produce alternaric acid in synthetic culture media produces a substance with similar biological properties when inoculated into tomato fruits. Since other workers have shown that some of the symptoms of *A. solani* attack are toxigenically induced, it is tempting to suggest that alternaric acid is the toxin concerned. On the other hand, there is no correlation between virulence of strains of *A. solani* and their ability to produce alternaric acid in synthetic culture media or in tomato fruits. Some highly virulent strains produce little or no alternaric acid under any conditions tested. There is some evidence that other toxins beside alternaric acid are produced by *A. solani*, not necessarily in the same proportions by all strains.

Alternaria solani causes 'early-blight' of potatoes and tomatoes. The disease is of world-wide distribution but, though very destructive in many countries, notably in the United States, it is at present unimportant in Great Britain (Moore, 1943, 1948). A characteristic feature of the disease is the production of black lesions on stems and leaves of affected plants, these lesions eventually becoming papery and brittle. Whole leaves may become desiccated, leading to complete defoliation. The fungus can usually be isolated from the black lesions but on occasion, more particularly when the primary infection is of the collar-rot type at the base of the stem of young plants, stem and leaf lesions appear to be sterile. Whipple (1938) and Thomas (1940) have supposed that in such cases the fungus has produced a toxin in the basal tissues of the plant and that this has travelled upwards within the plant, causing lesions in advance of the actual spread of the

fungus. Thomas (1948) has suggested that, in foliage infections of more mature tomato plants, the rapid necrosis of leaf tissues around a point of infection is most easily understood by supposing that the fungus produces a toxin within the leaf tissue. Pound & Stahmann (1951) have provided firm evidence that production of toxins in the host plant is characteristic of attack by *A. solani*.

In a preliminary communication Brian, Curtis, Hemming, Unwin & Wright (1949) described a metabolic product of *A. solani*, which they named alternaric acid. This substance is antifungal and phytotoxic, and they suggested that it might be the toxin postulated by Whipple and Thomas. The conditions of production of alternaric acid, and its antifungal properties, have been described in greater detail by Brian, Curtis, Hemming, Jefferys, Unwin & Wright (1951); the present communication deals with the phytotoxic properties of alternaric acid and with its part in the etiology of plant diseases caused by *A. solani*.

EXPERIMENTAL MATERIALS

The following are the code numbers and sources of the isolates of *A. solani* used in the experiments described below:

IBPG 169a, potato, England, 1949; IBPG 170, potato, South Africa, 1950; IBPG 171, potato, India, 1950; IBPG 174, potato, South Africa, 1950; IBPG 178, potato, India, 1950; IBPG 183, tomato, United States; IBPG 186, potato, Australia, 1950; IBPG 187, potato, United States, 1950; IBPG 188, potato, Australia, 1950; BRL 408, obtained from R. V. Tipler, Hawthorndale Laboratories, Jealott's Hill Research Station. This was a subculture from a strain (IBPG 14) isolated from tomato fruit, 1946, at the Imperial Bureau of Plant Breeding and Genetics, Cambridge. The nine isolates with IBPG numbers were supplied by Mr A. W. Charles, Commonwealth Potato Collection, Cambridge; they were all virulent isolates. BRL 408 is believed to be of very low virulence.

All alternaric acid used in these experiments was prepared from cultures of isolate BRL 408 by methods previously described (Brian *et al.* 1951).

The composition of the two media used in some experiments has been previously given in detail. Both have a similar mineral salt content, both contain 10% sucrose, but differ in nitrogen source, this being nitrate in medium N and casein hydrolysate in medium T.

PHYTOTOXIC EFFECTS OF ALTERNARIC ACID

(J. M. Wright)

Germination tests

Phytotoxicity was first assessed by a simple germination test. Seeds of various plants were germinated on agar (at two pH levels) containing alternaric acid at several concentrations and, after a suitable interval (usually *c.* 6 days at 25° C.), percentage germination and the amount of root development were measured. The technique has been described in greater detail by Wright (1951). The results

presented in Table 1 show that alternaric acid was toxic at low concentrations to all the seeds tested, though the different species differed in their sensitivity. Alternaric acid usually had a greater effect on root growth than on percentage germination. The pH of the medium had no marked effect on the results.

TABLE 1. *The effect of alternaric acid on percentage germination of seeds and root development (expressed as percentage of untreated controls)*

Plant		Germination (%) Alternaric acid ($\mu\text{g./ml.}$)				Root development Alternaric acid ($\mu\text{g./ml.}$)		
		25	5	1	0	25	5	1
Clover	<i>a</i>	77	98	100	98	17	89	105
	<i>b</i>	67	97	98	100	15	73	86
Wheat	<i>a</i>	90	98	100	98	10	59	95
	<i>b</i>	90	98	100	96	10	55	95
Carrot	<i>a</i>	0	29	69	75	0	15	96
	<i>b</i>	4	35	75	75	0	20	71
Mustard	<i>a</i>	38	85	87	92	0	10	29
	<i>b</i>	43	80	93	88	0	16	45
Radish	<i>a</i>	40	81	88	98	0	15	37
	<i>b</i>	37	69	86	90	0	15	45
Tomato	<i>a</i>	0	8	94	94	0	1	56
	<i>b</i>	0	6	71	88	0	0	34

a: pH 4.0. *b*: pH 6.0.

Experiments on cut shoots

Potato and tomato

Cut shoots of potato (var. Arran Pilot) were placed in tap-water solutions of alternaric acid (2, 5, 10 and 20 $\mu\text{g./ml.}$) and compared with similar shoots in tap water only. After about 45 hr. black patches appeared on the edges of the leaflets of shoots in the highest concentration of alternaric acid; these lesions were mostly on the lower leaves. A few hours later these black patches had increased in size, necrosis had appeared in intercostal areas and long dark necrotic lesions appeared on stems and petioles; the shoots in 10 $\mu\text{g./ml.}$ alternaric acid developed similar symptoms at this time. Though the leaf-blades became severely wilted, stems and petioles remained turgid. In succeeding days the shoots in each of the concentrations of alternaric acid developed similar symptoms, and on the seventh day all leaves were desiccated and dead, but the stems and petioles remained turgid, though bearing typical necrotic streaks. Lesions on leaves and petioles are illustrated in Pl. 13, figs. 1 and 2.

Affected stems were sectioned, and it was found that the tissues most affected were the collenchymatous subepidermal layers. Here the cell walls and cell contents had become dark brown. Though plugged and discoloured xylem elements were occasionally seen, damage to the xylem was not a characteristic symptom.

The lesions produced were similar in shape and colour to those described and illustrated in standard text-books (e.g. Viennot-Bourgin, 1949; Walker, 1950) as

characteristic of *A. solani* attack, though lesions of the 'target-spot' type were not seen. A sample of naturally diseased potato haulm sent to us from Devon by Dr G. C. Ainsworth showed lesions almost indistinguishable from those produced by alternaric acid.

Similar effects were produced on cut shoots of tomato (var. Potentate). This plant was considerably more sensitive than potato, a concentration of 0.25 µg./ml. being sufficient to desiccate and kill the leaves.

The sequence of symptoms of alternaric acid toxicity to potato and tomato are as follows: (i) black lesions on leaf-blades, characteristically on the edges of the leaflets or in intercostal areas of the leaf; (ii) elongated black or dark brown necrotic lesions on the stems and petioles; (iii) wilting, followed by desiccation of the leaf-blades without noticeable loss in turgor of the stems and petioles. Before the first dark lesions on the foliage appear, a change occurs in the appearance of the epidermis; the epidermal cells appear to collapse in places and this is followed by the development of water-soaked spots which rapidly become dark in colour.

Effect on other species

Alternaric acid affects a far wider range of species than the Solanaceous plants providing the normal host-range of *A. solani*. Cut shoots of a number of plants were placed in alternaric acid solutions. *Atropa bella-donna* L., *Solanum dulcamara* L., *Urtica dioica* L. and *Chamaenerion angustifolium* (L.) Scop. rapidly developed typical lesions. Lesions were also produced, more slowly, on *Betula pendula* Roth and *Impatiens* sp. No lesions were produced on *Bambusa* sp., but this was thought to be due to poor water uptake. In a series of experiments on the effect of alternaric acid on plants growing in water culture, it was found to be toxic to radish, carrot, mustard and cabbage.

Tissue specificity of the response to alternaric acid

The effects produced by alternaric acid, described above, are to some extent specific. This can be seen in a comparison with the effects produced by patulin (clavacin), a phytotoxic antibiotic studied by Gäumann & Jaag (1947*b*). Patulin is more generally toxic than alternaric acid; it is toxic to a wider range of fungi and, unlike alternaric acid, is markedly antibacterial. It is less phytotoxic than alternaric acid; in our experience concentrations below 20 µg./ml. have little effect on cut shoots. A comparison of the effects produced on potato shoots by alternaric acid (20 µg./ml.) and patulin (100 µg./ml.) is shown in Pl. 13, fig. 3. Alternaric acid-treated shoots show the characteristic retention of turgor by stems and petioles, whereas shoots in patulin show a general collapse. Leaf lesions produced by alternaric acid appear in the intercostal areas of the leaf (Pl. 13, fig. 4) but those produced by patulin appear first in the veins and adjacent tissues (Pl. 13, fig. 5). Thus plant tissues show a definite specificity in their response to alternaric acid;

whereas patulin damages all tissues through which it passes, alternaric acid passes through some tissues (e.g. the veins of the leaf and the conducting zones of the stem) without causing damage.

Experiments on water balance

A characteristic symptom of alternaric acid phytotoxicity is desiccation of the foliage. An investigation of the effects of alternaric acid on the water balance of cut shoots was therefore undertaken. Water absorption was measured by a potometer and transpiration by weighing. Readings were made as often as necessary, usually every 30 min. in experiments lasting about 60 hr.

Shoots of *Atropa bella-donna*, which have a smooth, firm stem, easily fitted in a potometer without crushing, and being highly sensitive to alternaric acid, were used in all experiments. The shoots were cut in the evening and left in distilled water in the experimental room overnight so that they might reach water equilibrium. Early next morning shoots of approximately the same size and age were chosen and fixed in potometers filled with air-free distilled water. They were left for 3-4 hr. to recover from any shock caused by handling, and the water was then removed and replaced by appropriate alternaric acid solutions. Shoots were weighed before and after the experiment. The water loss so determined served as a check on the measurements made during the course of the experiment. Observations were made on the time of appearance of leaf lesions, wilting and desiccation.

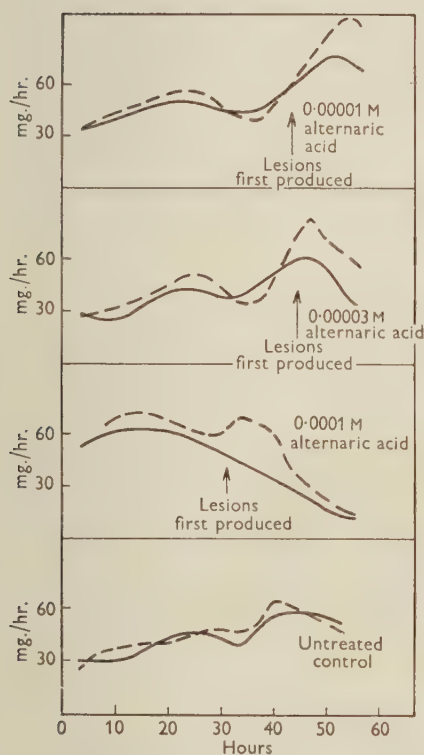
TABLE 2. *The effect of alternaric acid on uptake and loss of water by Atropa shoots: protocol of an experiment at constant temperature (25° C.). (Duration of experiment, 57 hr.)*

Conc. of alternaric acid (M)	Young shoots				Mature shoots		
	0	0·0001	0·00003	0·00001	0	0·0001	0·00003
Initial fresh weight (g.)	36·7	49·9	34·7	45·1	20·0	24·5	21·2
Fresh weight loss (%)	0·6	43·7	28·4	22·8	22·5	38·4	36·4
Water taken up (%)	239·8	262·4	231·5	295·0	401·5	342·0	460·0
Water transpired (%)	247·0	310·5	272·0	320·5	422·5	382·9	497·5
Water deficit (%)	7·2	48·1	40·5	25·5	21·0	40·9	37·5
Time taken for first lesions to appear (hr.)	—	31·9	43·5	43·6	—	29·0	31·4
Water deficit percentage on first appearance of lesions	—	6·4	12·1	3·1	—	9·4	9·4

Note. Percentages are in terms of initial fresh weight.

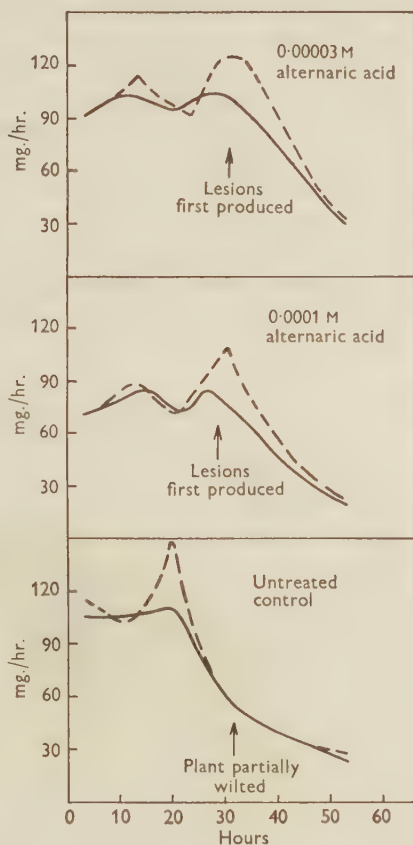
Some experiments were carried out at a controlled temperature of 25° C., others at the variable temperature of the laboratory. No attempt was made to control atmospheric humidity, but in the constant temperature experiments humidity was in fact virtually constant during each experiment. Experiments were carried out in duplicate or triplicate. Occasionally leaks developed in a potometer, or plants wilted as a result of injury; in such cases results were discarded.

Table 2 summarizes an experiment in which alternaric acid concentrations of 0.0001 (41 $\mu\text{g./ml.}$), 0.00003 and 0.00001 M were compared with water controls. Two types of shoot were used: (a) young flowering shoots with only a few berries



Text-fig. 1.

Text-fig. 1. The effect of alternaric acid on the rates of water uptake and transpiration of young *Atropa* shoots. (Rates are expressed as mg./hr./g. fresh weight of shoots. Full line = uptake; broken line = transpiration.)



Text-fig. 2.

Text-fig. 2. The effect of alternaric acid on the rates of water uptake and transpiration of mature *Atropa* shoots. (Rates are expressed as mg./hr./g. fresh weight of shoots. Full line = uptake; broken line = transpiration.)

formed, (b) more mature shoots, past the flowering period, bearing many berries. It will be noted first that uptake and loss of water per unit of fresh weight was much greater in the mature shoots than in the young shoots. As expected, the most obvious effect of alternaric acid was to cause a very considerable increase in the

net loss of water. There is an indication that alternaric acid had the effect of increasing water uptake per unit of fresh weight, but the data from this experiment alone would scarcely warrant this view. There is little doubt that transpiration per unit of fresh weight was increased, and as this increase exceeded any increase in water uptake, a net loss of water resulted. Lesions first appeared, as small water-soaked spots, 29–44 hr. after the beginning of the experiment. The water deficit in such plants at that time was relatively small, the greater part of the net loss of water occurring after the lesions appeared. It seems probable therefore that the appearance of lesions is not due to a disturbance in water balance but that both lesions and the loss of water balance are caused by some common functional failure. Support for this view can be found in the fact that the water deficit in control plants at the same time was 8.7% for the young shoots, 18.0% for the mature shoots. These values are larger than some of those in plants treated with alternaric acid, but the young shoot showed no pathological symptoms and the mature shoot showed only a slight loss of turgor.

TABLE 3. *The effect of alternaric acid on uptake and loss of water by Atropa shoots: protocol of an experiment at variable temperature (19–32° C.). (Duration of experiment, 60 hr.)*

Conc. of alternaric acid	0	0.0001 M		0.00003 M	
		Replicate 1	Replicate 2	Replicate 1	Replicate 2
Initial fresh weight (g.)	32.8	37.3	18.5	30.3	28.0
Fresh weight loss (%)	0.3	26.3	31.5	25.7	36.4
Water taken up (%)	290.8	429.0	480.4	511.5	339.0
Water transpired (%)	295.0	463.5	514.5	539.7	380.0
Water deficit (%)	4.2	34.5	34.1	28.2	41.0
Time taken for first lesions to appear (hr.)	—	31.9	36.5	38.5	38.5
Water deficit percentage on first appearance of lesions	—	6.4	5.4	11.9	14.3

Note. Percentages are in terms of initial fresh weight.

Rates of water uptake and transpiration in these same experiments are shown in Text-figs. 1 and 2. A difference can again be seen between young (Text-fig. 1) and mature (Text-fig. 2) untreated shoots. Water uptake and loss by young shoots tended to increase slightly in rate during the course of the experiment whereas the mature shoots, after a preliminary stage of steady uptake and increasing water loss, entered a phase of rapid decline in rate of uptake and rate of loss of water. Alternaric acid (0.0001 and 0.00003 M) had no obvious effect on the rate of water uptake of mature shoots though the rate of water loss (transpiration) was temporarily increased, particularly in the period after the appearance of leaf lesions. The same may be said of the effect of the two lower concentrations (0.00003 and 0.00001 M) of alternaric acid on young shoots; the higher concentration (0.0001 M) caused a very early decline in rate of water uptake.

The results of an experiment at normally fluctuating summer temperature (19–32° C.) are presented in Table 3. Rate data are not presented in this case because temperature fluctuations obscured other effects. The same general conclusions can be drawn. The water deficit of plants in alternaric acid was greatly increased. In this experiment there was stronger evidence that alternaric acid increased the amount of water taken up per unit fresh weight. The amount transpired was even more increased, this accounting for the net loss. Again, the water deficit when lesions on the foliage appeared was quite low and of the same order as that observed in controls at the same time.

A somewhat similar investigation of the effects of lycomarasmin, a toxin produced by the tomato wilt pathogen, *Fusarium lycopersici*, and of patulin, an antibiotic produced by *Penicillium expansum*, has been described by Gäumann & Jaag (1947*a, b*). Alternaric acid resembles lycomarasmin more closely than patulin, both in the actual lesions it produces and in the details of its effects on water-balance.

THE RELATION BETWEEN ALTERNARIC ACID PRODUCTION AND PATHOGENICITY OF *Alternaria solani*

(P. W. Brian, G. W. Elson and H. G. Hemming)

The data so far presented demonstrate that alternaric acid can produce effects on plants in many respects similar to those occurring in diseases caused by *A. solani*. It would be easy to conclude that production of alternaric acid by *A. solani* in host tissues is largely responsible for many of the symptoms of the disease. Before this can justifiably be done, answers to the following questions are needed. Can *A. solani* produce alternaric acid in plant tissue? Do all strains of *A. solani* produce alternaric acid? Do highly virulent strains of *A. solani* produce more alternaric acid than less virulent strains? Experimental results bearing on these questions are presented below.

Production of alternaric acid in plant tissues

Three ripe tomato fruits were inoculated laterally with mycelium of *A. solani* (BRL 408), the superficial wound in which the inoculum was placed being subsequently sealed with paraffin wax. Three similar fruits were treated in the same way but no inoculum was inserted in the wound. All operations were carried out under aseptic conditions. The fruits were incubated at 25° C. for 10 days. Small dark rots (*c.* 1.5–2 cm. diam.) were produced in the inoculated fruits but the uninoculated controls remained sound. Each fruit was then divided tangentially into three segments, A including the tissue nearest the point of inoculation, C including the tissues most remote from the point of inoculation, and B those tissues intermediate between A and C. The juice from each portion was expressed, filtered and assayed for alternaric acid by a modified *Botrytis allii* spore germination

technique (Brian *et al.* 1951) in which the stunting effect on germ-tubes was compared with the effects in a dilution series of a standard alternaric acid solution. The estimated concentrations of alternaric acid present are given in Table 4. This assay is not specific for alternaric acid, but it seems highly probable that in this experiment the results reliably indicate its production. Significant amounts were produced under these conditions and some diffused to tissues distal to the rot.

TABLE 4. *Estimated concentrations ($\mu\text{g./ml.}$) of alternaric acid produced in tomato fruits inoculated with *Alternaria solani*, after incubation at 25° C. for 10 days*

	Inoculated			Uninoculated		
	A	B	C	A	B	C
Replicate 1	0.8	0.4	0.02	0	0	0
Replicate 2	0.8	0.2	0.02	0	0	0
Replicate 3	1.6	0.04	0.01	0	0	0

*Production of alternaric acid by strains of Alternaria solani
varying in origin and virulence*

Ten strains of *A. solani* were grown on two liquid media previously found (Brian *et al.* 1951) to be optimal for production of alternaric acid by strain BRL 408. These strains varied in origin, collections from England, South Africa, India, Australia and the United States being included; they also varied in pathogenicity (see Table 7), those strains with IBPG numbers being more pathogenic than BRL 408. Table 5 shows the development of 'alternaric acid activity' in culture filtrates; 'alternaric acid activity' is expressed as the number of twofold serial dilutions in which *Botrytis allii* germ-tubes show a certain arbitrary degree of stunting (Brian *et al.* 1951). This activity index is thus related to the logarithm of the concentration of alternaric acid or any other substance producing a similar physiological effect.

Strain BRL 408 produced a much greater effect than any of the other strains. The highest titre obtained with BRL 408 (activity index: 15) corresponds to a concentration of alternaric acid 128 times greater than the highest titre obtained with any other strains (activity index: 8 for IBPG 183 on medium N). Extraction experiments with a number of these strains have failed to show any production of alternaric acid, though other substances causing 'stunting' of *B. allii* germ-tubes have been obtained in small yield (Curtis, 1951). This experiment strongly suggests that alternaric acid is not produced by all strains of *Alternaria solani*. It remains possible that other phytotoxic substances might be produced.

In Table 6 results are given in which culture filtrates from all strains, growing on medium N with 10% sucrose, were tested for phytotoxicity to cut shoots of *Atropa bella-donna* as well as for the stunting effect of *Botrytis allii* germ-tubes. The antifungal data confirm those given in Table 5. Three kinds of phytotoxic

action were seen: (i) lesions on stem and leaves, without loss of turgor in petioles and stems, characteristic of alternaric acid toxicity; (ii) complete permanent wilting with no development of necrotic lesions; (iii) a combination of the two former actions, in which plants showing alternaric acid type lesions also exhibited some degree of permanent wilting of stems and petioles as well as leaves. Only four of the highly pathogenic strains (IBPG 174, IBPG 178, IBPG 186 and IBPG 187) gave unequivocal indication of the production of toxins acting similarly to alternaric acid, and these were less effective than culture filtrates from the weakly pathogenic strain BRL 408.

TABLE 5. 'Alternaric acid activity index' in cultures of ten strains of *Alternaria solani* on two media (N and T) (10% sucrose: initial pH 6.5) as determined by *Botrytis allii* bioassay

		Days growth at 25° C.					
		6	10	14	18	21	25
		Activity index					
N	IBPG 169a	0	3	2	6	6	4
	IBPG 170	0	2	0	0	3	1
	IBPG 171	0	4	1	0	2	3
	IBPG 174	0	1	4	2	3	4
	IBPG 178	0	0	4	3	5	5
	IBPG 183	0	1	8	6	4	0
	IBPG 186	0	5	2	3	3	3
	IBPG 187	0	1	5	0	1	4
	IBPG 188	0	2	1	1	2	6
	BRL 408	0	5	10	11	11	13
T	IBPG 169a	0	5	4	8	5	5
	IBPG 170	0	3	3	2	5	1
	IBPG 171	0	3	3	2	5	1
	IBPG 174	0	4	5	7	2	3
	IBPG 178	0	4	3	3	6	3
	IBPG 183	0	5	4	0	3	3
	IBPG 186	3	2	3	2	2	3
	IBPG 187	2	4	6	3	3	4
	IBPG 188	2	4	4	3	2	4
	BRL 408	7	8	12	13	13	15

It might be objected that the synthetic culture medium used in these experiments favoured strain BRL 408, and that in host tissues the greater invasive powers of the more highly pathogenic strains might enable them to produce more toxin. This possibility has not been fully explored but the experiment illustrated by Table 7 indicates that, though the IBPG strains grow more rapidly on tomato fruits than BRL 408, they still produce little or no alternaric acid or other substances with a similar action on *B. allii*. In this experiment four tomato fruits were inoculated laterally with each of ten strains of *Alternaria solani*. The diameter of the rotted lesion produced was measured after 14 days' growth at room temperature, and the juice was expressed from the half of the fruit nearer the point of inoculation and

assayed for alternaric acid by the *Botrytis allii* bioassay. Strain BRL 408 produced the smallest rot but produced the greatest *B. allii* germ-tube stunting activity in the expressed juice. It is true, nevertheless, that its superiority in production of antifungal substances was less marked than on synthetic media.

TABLE 6. *Antifungal activity (Botrytis allii bioassay) and phytotoxicity to Atropa shoots of culture filtrates from ten strains of Alternaria solani on medium N (10% sucrose; initial pH 6.5)*

Strain	Age of culture (days)	Antifungal activity index	Phytotoxicity of <i>Atropa</i> . Dilution of filtrate		
			Undiluted	1 in 4	1 in 16
IBPG 169a	14	3	W	W	
	21	3	W	W	W
IBPG 170	14	1	W	W	
	21	1	W	W	W
IBPG 171	14	3	W	o	
	21	3	W	o	o
IBPG 174	14	1	WA	A	
	21	o	W	A	o
IBPG 178	14	1	W	A	
	21	2	WA	A	o
IBPG 183	14	o	WA	o	
	21	o	WA	o	o
IBPG 186	14	2	o	o	
	21	3	A	A	o
IBPG 187	14	2	A	A	
	21	3	WA	A	o
IBPG 188	14	2	WA	WA	
	21	2	W	W	W
BRL 408	14	12	WA	A	
	21	14	A	A	A
Culture medium control		o	o	o	o

W=wilting of leaves, petiole and stem; A=alternaric acid type lesion; o=no effect.

TABLE 7. *Rate of growth of strains of Alternaria solani in tomato fruits and antifungal activity index (Botrytis allii bioassay) of the expressed juice after growth of the fungus (14 days)*

Strain	Mean diameter of rot (mm.)	Mean antifungal activity index
IBPG 169a	29	2
IBPG 170	34	o
IBPG 171	42	o
IBPG 174	39	1
IBPG 178	36	1
IBPG 183	35	o
IBPG 186	37	1
IBPG 187	33	1
IBPG 188	35	1
BRL 408	21	3
Uninoculated controls	o	o

The main conclusions to be drawn from these results are: (i) alternaric acid is not produced on synthetic culture media or on tomato fruits by all strains of *Alternaria solani*; (ii) other phytotoxic substances are almost certainly involved; (iii) capacity to produce alternaric acid or other phytotoxic substances is not directly related to the pathogenicity of any given strain.

DISCUSSION

We are now in a position to consider more confidently whether production of alternaric acid by *A. solani* is important in the etiology of plant diseases caused by that organism. The evidence in favour of this supposition is, briefly: (i) Alternaric acid, if introduced systemically into plants, produces lesions very similar to those found in certain phases of the disease produced by infection with *A. solani*. (ii) Alternaric acid is produced by some strains of *A. solani* in synthetic culture media and there is strong evidence that it is produced in ripe tomato fruit tissue and, therefore, quite possibly also in other plant tissues. (iii) There is little doubt, especially in view of the evidence of Pound & Stahmann (1951), that *A. solani* produces a toxin in the tissues of host plants. It must be noted that this evidence, however convincing, is only circumstantial and that there is no direct evidence that alternaric acid is the toxin produced in naturally diseased plants; Gäumann (1951) has noted that similar qualifications apply when discussing the role of lycomarasmin in the etiology of the tomato-wilt produced by infection with *Fusarium lycopersici*.

On the other hand, the following facts have to be considered:

(i) Of the twelve strains of *Alternaria solani* which we have studied (this paper and Brian *et al.* (1951)), only two have been definitely shown to produce alternaric acid, and though more refined methods might have detected traces of alternaric acid production by other strains, it is certain that the amount produced, if any, is very much less than that produced by the two active strains (BRL 407 and BRL 408). However, alternaric acid-producing strains of *A. solani* have been found by other investigators. In investigations independent of but simultaneous with ours, Pound & Stahmann (1951), working at Wisconsin, have also isolated alternaric acid from highly phytotoxic culture filtrates of *A. solani*, and have assigned to it a major role in the development of early blight symptoms. Darpoux, Faivre-Amiot & Roux (1950) have isolated a substance which is probably alternaric acid from French strains of *A. solani*.

(ii) The two strains which we have found to produce alternaric acid are the least pathogenic of those studied.

(iii) There is evidence, both from our work and that of Pound & Stahmann (1951) that other phytotoxic substances are involved. Gäumann (1951) reports a similar situation in wilts produced by *Fusarium lycopersici*. Of two strains of *F. lycopersici*, one which is almost non-pathogenic produces large yields of the wilting-factor lycomarasmin, whereas the other, highly pathogenic, produces very little. He suggests that the metabolism of the fungus may be very different in host tissues

and that in the host the pathogenic strain may be able to produce more lycomarasmin than the non-pathogenic strain. We have considered this possibility in connexion with *Alternaria solani* and, on the basis of experiments in which *A. solani* was grown on tomato fruit, admittedly different from stem or leaf tissue, were forced to conclude that this could not explain the apparent lack of correlation between pathogenicity and capacity to synthesize alternaric acid.

These results have some bearing on the prospects of alleviating toxigenically induced plant diseases by inactivating the toxin *in vivo*. Howard (1941) has reported a reduction in the severity of attack by *Phytophthora cactorum* in maple shoots by systemic treatment of the plant with diaminoazobenzene hydrochloride. Horsfall & Zentmyer (1941) have reported similar results, using the same compound, in *Ceratostomella ulmi* attack on elm shoots (Dutch elm disease). In both cases there was some evidence that the disease control was obtained by neutralizing the toxins produced rather than by a more conventional fungicidal or fungistatic effect. As yet we have insufficient experience of this method of control to be sure that such treatments reduce disease severity with all strains of the fungi concerned. Albert (1951) has suggested that glutathione, known to inactivate lycomarasmin, would be used as a systemic treatment of tomato *Fusarium* wilt, were it not so costly. As we have seen, there is grave doubt whether lycomarasmin is produced by all pathogenic strains of *Fusarium lycopersici* and, consequently, glutathione treatment might not be so universally applicable as Albert suggests. Similarly, it might be possible, more particularly when more is known of the chemistry of alternaric acid, to find an 'antidote' which could be introduced systemically into plants to prevent many of the symptoms of early blight. But, as we have seen, alternaric acid production is characteristic of certain strains only of *Alternaria solani* and an inactivator of alternaric acid would only be effective in disease caused by these strains. It is not intended to deny the usefulness of the principle of disease control by systemic toxin-inactivation, but the results presented here show that disease control by such means may not prove so straightforward or so effective as is sometimes thought.

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EXPLANATION OF PLATE 13

- Fig. 1. Lesions produced on potato leaflets taken from cut shoots placed in alternaric acid solution (2 µg./ml.).
- Fig. 2. Lesions produced on potato stem of cut shoot placed in alternaric acid solution (2 µg./ml.).
- Fig. 3. Right: potato shoot after 6 days in alternaric acid solution (20 µg./ml.). Left: potato shoot after 6 days in patulin solution (100 µg./ml.).
- Fig. 4. Lesions produced in tomato leaflet taken from cut shoot placed in alternaric acid solution (0.25 µg./ml.).
- Fig. 5. Lesions produced in tomato leaflet taken from cut shoot placed in patulin solution (20 µg./ml.).

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DRY-ROT DISEASE OF THE POTATO

IV. LABORATORY METHODS USED IN ASSESSING VARIATIONS IN TUBER SUSCEPTIBILITY

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(With Plate 14)

A laboratory method is described in which a modified hypodermic syringe is used to inoculate potato tubers with *Fusarium caeruleum*. This injection method gives consistent results and permits reliable assessments of factors such as varietal susceptibility.

The occurrence of arrested lesions is noted.

Suspensions of very high spore concentration or of spores already germinated did not greatly increase the amount of dry-rot above that given by the standard 100 spores per inoculation, provided that spore germination was adequate.

The size of tuber and site of inoculation were found to have a considerable effect upon the results of inoculation experiments, large tubers being more susceptible than small ones and the heel end being more susceptible than the rose end. The necessity for uniformity of material used in inoculation experiments is emphasized.

It is well known that potato varieties differ considerably in their reaction to the dry-rot fungus *Fusarium caeruleum* (Lib.) Sacc. It is known also that certain other factors, such as the time of year, alter the susceptibility of the tubers even within a variety. The development of a reliable method of testing for susceptibility is highly desirable, especially for new varieties. Before such a method can be utilized with the confidence that the results obtained are not only reliable but are capable of correct interpretation, as many as possible of the factors influencing susceptibility must be studied. This and the following three papers describe variations brought about by a number of such factors as examined by a standard inoculation technique.

In 1917, Pethybridge & Lafferty described a method of artificial inoculation of tubers which consisted of taking out a small core of tissue with a sterile scalpel, placing the inoculum at the base of the cavity so produced and replacing the core of tissue after cutting off the apex. The inoculated tubers were kept in covered glass dishes at laboratory temperature. They suggested that a systematic susceptibility test of commercial varieties might yield economic results. Moore (1945) used the method of inoculating cylindrical wounds approximately 6 mm. in diameter and 6 mm. deep made by a metal rod cut down at one end to these dimensions. The inoculated tubers were kept in boxes lined with damp cotton-wool and covered for

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the first month. In France, Lansade (1949) has recently described a method of inoculation by cutting whole tubers after sterilization and applying a spore suspension to the cut surface. The tubers were then incubated in glass jars at laboratory temperature.

MATERIALS AND METHODS

Standard laboratory injection method

The inoculum. For uniformity, a spore suspension of the fungus calibrated to a known density was prepared from a 2 to 3-week culture of a monospore isolate of the fungus growing on potato dextrose agar. In preliminary experiments it was found that repeated subculturing tended to produce loss of pathogenicity: consequently, in most of the experiments described the isolate used was inoculated into sterilized soil which was then allowed to become air dried (Miller, 1946). To produce the spores for inoculation, a little of the soil was shaken on a freshly poured plate of potato dextrose agar which, after incubation, produced sporodochia in about 10 days. The spore suspension was made up in tap water, filtered through muslin and calibrated using a haemocytometer slide. Suspensions giving counts of about 90–120 per inoculation droplet gave reproducible results.

Method of inoculation. In early experiments a glass inoculator (Foister, Wilson & Boyd, 1945) was used to wound the tubers, and the spore suspension was applied with a platinum loop. To carry out wounding and inoculation in one action, an inoculator was developed from a 1 ml. hypodermic syringe (Pl. 14, fig. 1). The needle in the needle block was cut off and the hole soldered, while another hole 1 mm. in diameter was bored in the side of the needle block 2 mm. from the end. The tuber could thus be wounded to a uniform depth of 7 mm. by forcing the needle block into the tissue, while the shoulder of the block prevented further penetration (Pl. 14, fig. 2). The syringe was fitted with a screw dosage regulator, one half turn of which was made for each inoculation and the spore suspension was so calibrated that the quantity ejected ($\frac{1}{7\frac{1}{4}}$ ml.) contained on the average the standard 100 spores. In practice the hole rarely became blocked by tuber tissue, but could easily be cleared with a pin.

The tubers. All tubers used were carefully lifted and immediately surface-sterilized in 2% formalin for $\frac{1}{2}$ min. They were then dried and kept until required in large paper sacks in a potato store, care being taken that no chilling occurred during cold weather. Twenty tubers were used for each variety of potato or other variable factor under investigation, each tuber being inoculated on opposite faces, approximately half-way between rose and heel ends, giving forty inoculations for each sample. In preliminary experiments similar numbers of control tubers were wounded and treated in the same way as the inoculated tubers except that no inoculum was applied. It was found, however, that since the tubers had been originally surface-sterilized extraneous contamination was quite negligible so no further control tubers were used in the later experiments.

Incubation. After inoculation the tubers were placed in cardboard boxes containing a layer of damp peat (Pl. 14, fig. 4). Two boxes were used per sample, i.e. ten tubers per box. These were stacked in rows on wooden slats in a chamber (Pl. 14, fig. 3) consisting of a wooden shell approximately $5 \times 5 \times 5$ ft., designed to fit as closely as possible over a 5×5 ft. zinc tray. The front of this chamber formed a door and all the inside walls were lined with sacking. The zinc tray contained a 1 in. layer of damp peat which, together with the sacking, was sprayed with water periodically to maintain the high relative humidity especially necessary during the first 10 days after inoculation. Over a period of 6 months the R.H. averaged 94 % with a range of from 88 to 96 %. The whole chamber was kept in a centrally heated room maintained at between 52 and 60° F., the average temperature being 57° F. which is near the optimum for infection (Moore, 1945).

An attempt was made to reduce the space necessary for the incubation of large numbers of tubers by inoculating tangential tuber slices with a drop of standard spore suspension and storing them in trays in a covered zinc box at about 15° C. The results obtained were generally in agreement with those given by the inoculator method but tended to be more erratic.

Method of assessment. Generally, all tubers were cut through the points of inoculation after incubation for 5–6 weeks. Only where active rotting had occurred was the lesion counted as positive. In some cases the diameter and depth of such lesions were measured, and records kept of the numbers of inoculations where, although no rotting was produced, necrosis of the tissue to an extent of more than 10 mm. in diameter was caused.

EXPERIMENTAL RESULTS

(1) *Reliability of the standard method.* Normally twenty tubers, each inoculated in two places, were used in each test and stored during incubation in two cardboard boxes each with ten tubers. These two boxes (A and B) were then treated as replicates, placed at random in the incubation chamber and recorded separately. Table 1 shows the numbers of positive inoculations (maximum 20) in each box for a series of twenty-three varieties at four different times during the storage season. In each entry the higher figure, if any, has been placed first. The figures for the replicate boxes are clearly so close as scarcely to warrant statistical proof of significance of the general reliability of the method. The overall mean difference between the two replicates of the ninety-two sets inoculated in this series was 1.5. Varietal reaction will be discussed in another paper.

(2) *Arrested lesions and necrosis.* Frequently, where no advancing rot surrounded the inoculation mark after 6 weeks storage, the appearance of the surrounding tissues indicated that the fungus had penetrated for several millimetres, then had ceased to advance and a wound periderm had sealed off the area. The fungus was frequently recovered from the necrotic tissues in such lesions where it apparently remained dormant. To determine whether such lesions could become active again

half of the tubers in one experiment were left uncut after examination and incubated for a further 6 weeks. When the uncut tubers were finally examined after the further incubation only eight out of an estimated total of 200 arrested lesions had become active. Some of these may have been progressing very slowly and may not have been obvious on the first examination. It would appear that there is no material advantage in incubating tubers under the standard conditions any longer than 6 weeks after inoculation since such rotting as does develop does not appreciably affect the final figure.

TABLE I. *Numbers of positive inoculations (maximum 20) in two replicate boxes (A and B) of twenty-three varieties and four inoculation dates*

Variety	Inoculation 1			Inoculation 2			Inoculation 3			Inoculation 4		
	A	B	Difference	A	B	Difference	A	B	Difference	A	B	Difference
1	0	0	0	1	1	0	0	0	0	20	18	2
2	10	9	1	6	6	0	6	6	0	20	20	0
3	5	2	3	3	1	2	6	4	2	20	20	0
4	2	1	1	12	11	1	3	2	1	20	20	0
5	9	5	4	9	8	1	6	2	4	20	20	0
6	2	0	2	17	14	3	3	0	3	18	16	2
7	6	4	2	1	1	0	6	5	1	20	20	0
8	0	0	0	3	2	1	4	2	2	19	18	1
9	0	0	0	13	11	2	4	3	1	20	20	0
10	2	2	0	12	10	2	6	5	1	19	19	0
11	0	0	0	17	15	2	10	9	1	20	20	0
12	11	9	2	0	0	0	11	7	4	20	20	0
13	0	0	0	1	0	1	0	0	0	14	8	6
14	0	0	0	14	11	3	1	0	1	18	17	1
15	4	2	2	14	9	5	5	5	0	18	14	4
16	7	6	1	10	7	3	4	4	0	16	14	2
17	4	1	3	7	4	3	3	2	1	19	15	4
18	0	0	0	10	8	2	1	0	1	20	18	2
19	3	2	1	4	2	2	6	3	3	18	16	2
20	1	0	1	8	3	5	1	0	1	16	13	3
21	2	2	0	18	17	1	6	2	4	20	20	0
22	17	16	1	9	5	4	12	11	1	20	20	0
23	0	0	0	15	8	7	1	1	0	13	10	3

(3) *Spore load of inoculum.* Several experiments using a number of varieties of potato showed that within fairly wide limits the concentration of the spore suspension did not affect the resulting development of dry rot so long as the suspension contained more than about 50 viable spores per inoculation. Similar results were recorded by McKee & Boyd (1952). In a typical experiment, using Arran Pilot, 50, 100 and 200 spores per inoculation resulted in 87, 90 and 93 % dry rot respectively. Spore germination in this case was 74%. In another instance 30, 35 and 37.5 % resulted from inoculations of Arran Pilot respectively with 100 spores with an 82 % germination capacity, 800 spores with 97 % germination and 8000 spores with 11 % germination.

Comparison of the results given by standard spore suspensions and unstandardized mycelial or sporodochial inoculations revealed that the latter tended to provide a somewhat higher amount of dry rot. This is shown in Table 2.

TABLE 2. *Percentage dry rot produced in several experiments using sporodochial inoculations and standard spore suspensions*

Variety of potato	Home Guard	Doon Star	Arran Banner
Germination of standard suspension (%)	85	96	96
Dry rot produced by standard inoculation (%)	65	45	0
Dry rot produced by sporodochial inoculation (%)	75	57.5	2.5

The sporodochial inoculation was made by placing into a standard wound a small piece of the spore mass containing approximately 100,000 spores from the sporodochia produced on an agar culture. For general inoculation work, however, the standard spore suspension method was adhered to since its uniformity lessened the likelihood of any variable results and it served to distinguish differences in susceptibility of the tubers under test perhaps better than an extremely concentrated inoculum.

Germination of the spore suspension is obviously important, and a count was normally made 24 hr. after each suspension had been made up. Counts of over 75 % were considered satisfactory and these were generally obtained. It was found that a reduction in germination occurred corresponding to the density of the suspension as observed by Doran (1922) and by McCallan (1930) with *Botrytis allii* and *Cephalothecium roseum* but contrary to the observations of Blodgett (1936) with *Pseudopeziza ribis*. In one experiment hanging-drop suspensions averaging 503, 44 and 12 spores gave respectively 0, 19 and 44 % germination after 48 hr. at laboratory temperature. In another experiment 0.7 ml. of a very concentrated suspension containing about 700,000 spores per ml. was placed in a watch-glass. After 24 hr. counts were made in the field of a low-power microscope and in the centre of the watch-glass, where there were about 2000 spores per field, germination was 1 % while at the edge of the watch-glass where the concentration of spores was only about 100 per field germination was 65 %.

TABLE 3. *Percentage dry rot developing in different experiments after inoculation with germinated spore suspensions*

Variety of potato	Arran Pilot	Arran Consul	Arran Pilot	Doon Star	Arran Banner
Germination of suspension (%)	74	74	82	96	96
Dry rot (suspension ungerminated) (%)	90	48	30	45	0
Dry rot (suspension germinated 1 day) (%)	—	—	15	82.5	5
Dry rot (suspension germinated 2 days) (%)	70	60	57.5	—	—

It was thought that by the use of a standard suspension of germinated spores the fungus would be enabled to penetrate the tuber more quickly and perhaps overcome the defence mechanism of tubers of resistant varieties. Several experiments were conducted at different times and with different varieties with this in view but the results (Table 3) were variable, and although in some cases suspensions of germinated spores did lead to higher infection, it was only to a limited extent and it would not seem possible by this means consistently to overcome the resistance of a variety such as Arran Banner.

(4) *Site of the inoculation.* In all the experiments described inoculations were normally made in the sides of the tuber approximately midway between heel and rose end, care being taken as far as possible to avoid the eyes. Clear experimental evidence was, however, obtained that variations in susceptibility exist in different parts of the tuber. Forty standard inoculations were made in each case at the rose end, side and heel end of Doon Star tubers of a similar size and after incubation the amount of dry rot which developed was respectively 7.5, 37.5 and 42.5 %. In experimental work, therefore, it is obviously important to make the wounds in the same area of the tuber if comparable results are to be obtained.

(5) *Size of the inoculated tubers.* It is known that in practice after the ware tubers of a susceptible variety have been separated from the seed by riddling and are then reclassified dry rot frequently develops in them on an extensive scale. This may be naturally attributed to the heavier ware tubers sustaining more abrasions and allowing the fungus more avenues of entry. That tubers of different sizes however vary in inherent susceptibility was shown by an experiment where Doon Star tubers from a single stock were graded according to weight into five groups from $\frac{3}{4}$ to 8 oz. Twenty tubers from each group were inoculated in the standard way. Considerable differences were apparent in the results (Table 4) and indicate that on a weight basis small tubers are less susceptible to dry rot than large tubers and that, at least for the variety Doon Star, there is a marked change in reaction at the critical weight of between 4 and 5 oz.

TABLE 4. *Effect of size of Doon Star tubers upon the development of dry rot after inoculation*

Weight (oz.)	$\frac{3}{4}$ -1	1-2	2-4	4-5	5-8
Dry rot (%)	35	25	37.5	65	65

The dry-rot figure for the smallest category is higher than that for the next group and it is possible that in the smallest group another factor, that of relative depth of inoculation comes into play. In these tubers the inoculation penetrates relatively deeply and enters the central medullary zone. By comparing the reactions of tuber slices, in the method previously described, taken tangentially and also through the centre of potatoes of four varieties, it was found that the central pith zone tended to be more susceptible to rotting than the outer cortical layer. The mean figures for the four varieties were respectively 85 and 70 %. Lansade (1949) has also observed

that rotting takes place more rapidly in the central as compared with the cortical zones of the potato.

(6) *Greened tubers*. It is commonly stated that greened tubers are more resistant to dry rot than those which are ungreened, but in an inoculation experiment with the variety King Edward practically no difference in reaction was found. Where any difference is observed in practice it may be due to the resistance to abrasions conferred on greened tubers by reason of the tougher skin.

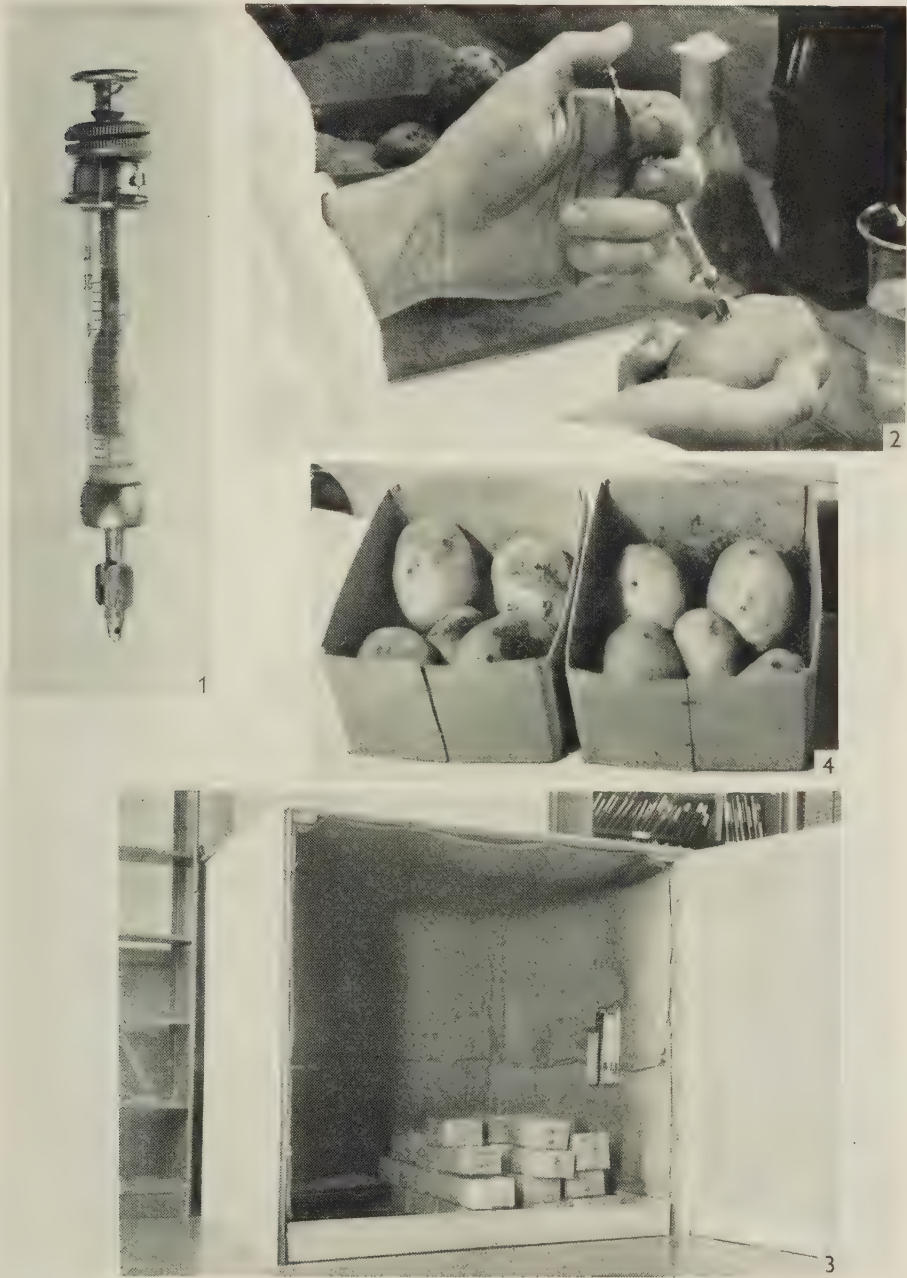
DISCUSSION

In the technique for inoculation of potato tubers with *Fusarium caeruleum* which has been described, the use of a spore suspension with a germination of 75 % or over and 100 spores per inoculation has been taken as a convenient standard. Variations of between half and many times this concentration of spores have only a very slight effect upon the amount of dry rot developing but a standard number is desirable in order to record the germination capacity of the spores, since in high concentrations a marked decrease in germination resulted in water suspensions. This has been attributed by Doran (1922) and others to a decreased oxygen concentration.

In contrast, however, it is much more important that the inoculated tissue should be as uniform as possible. In the standard inoculation the depth is uniform in all cases. It is conceivable, however, that if tubers of variable size are used different layers of tissue may be inoculated, e.g. in large tubers the inoculator penetrates only the cortex while in very small tubers the pith would be penetrated. It has been found in the present experiment, and also by Lansade (1949), that the central zone of the tuber is rather more susceptible than the cortex. This may account for a slightly increased dry-rot reaction in very small tubers ($\frac{3}{4}$ –1 oz. in weight) over those slightly larger (1–2 oz.). It is possible that the larger increases in susceptibility of tubers up to a critical weight of between 4 and 5 oz. are due to differences of composition of which there are several between tubers of different sizes. It is obvious that for a standard method, tubers of a uniform size must be used.

It is well known that different parts of a potato tuber vary in composition and this is especially so in the rose and heel ends. For example, the heel end normally contains higher percentages of dry matter and starch (Glynne & Jackson, 1919), 20–40 % more free tyrosin (Mulder, 1949), and less potash (Biewenga, 1929) than the rose end, and is usually more subject to internal discoloration after bruising (Boyd, 1951). It is not surprising therefore to find that the heel end and rose end vary so much in susceptibility to dry rot, although the explanation does not appear to be associated with any of the other variations. This fact again emphasizes the necessity for uniformity in the site of tuber inoculations in dry-rot experiments.

These investigations were carried out at the request of Dr T. P. McIntosh, Director of the Seed Testing, Plant Registration and Plant Pathology Station of the



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EXPLANATION OF PLATE 14

- Fig. 1. Modified hypodermic syringe used to inoculate tubers.
- Fig. 2. Method of inoculation.
- Fig. 3. Humidity chamber used for incubation of inoculated tubers.
- Fig. 4. Susceptible and resistant tubers after inoculation and incubation. Normally ten tubers are placed in each box and this closed by a lid.

(Received 27 December 1951)

DRY-ROT DISEASE OF THE POTATO

V. SEASONAL AND LOCAL VARIATIONS IN TUBER SUSCEPTIBILITY

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(With 2 Text-figures)

The standard dry-rot inoculation technique was employed to demonstrate variation in susceptibility in a number of commercial potato varieties over the whole storage season. Susceptibility is lowest at about normal lifting time and gradually rises. Different rates of seasonal progress in susceptibility between varieties were observed.

A very high peak of susceptibility occurs in immature tubers in the post-flowering period of plant growth, which, in the variety Doon Star, is in the latter part of August. This is followed by a sudden decrease which reaches a minimum when the haulm is dead. This susceptibility is not due to thinness of skin of the immature tubers but to physiological or chemical changes in the tubers.

Some differences in susceptibility were also observed in different stocks of the same variety, possibly due to soil conditions, but when samples of these stocks were grown on the same soil no clear differences were found in the overall susceptibility, although variations occurred at different sampling dates.

Pethybridge & Lafferty (1917) noted the effect of tuber maturity on susceptibility to *Fusarium caeruleum* which, they observed, was negligible when the tubers were lifted and at a maximum in early spring. Seasonal susceptibility was found also by Moore (1924), but Weiss, Lauritzen & Brierley (1928) observed in America no material difference in susceptibility to infection or rate of decay between recently dug tubers and those stored until the end of April. Recently Lansade (1949) has given further data on seasonal reaction to *F. caeruleum* in France and has indicated that different soils may influence tuber susceptibility. He also notes the susceptibility of tubers lifted in an immature condition.

MATERIALS AND METHODS

The tubers, unless otherwise stated, were grown in the same soil at East Craigs, surface sterilized in 2 % formalin at lifting time and kept before inoculation in large paper sacks in a potato store. The method of inoculation and incubation were as described in the previous paper (Boyd, 1952).

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EXPERIMENTAL RESULTS

(1) *Variation during storage.* During the period 1947-50, tubers of a number of varieties were inoculated at intervals from lifting time in October until planting time in the following April to determine seasonal susceptibility. The results of one

TABLE 1. *Percentage dry rot developing in nine potato varieties inoculated at monthly intervals in 1949-50*

Variety (in order of maturity)	Inoculation date						
	21 October	23 November	17 December	19 January	23 February	18 March	16 May
Epicure	0	0	2.5	5	17.5	30	100
Arran Pilot	27.5	40	42.5	85	82.5	82.5	100
Home Guard	7.5	17.5	27.5	40	45	65	95
Catriona	75	80	85	92.5	100	97.5	100
Majestic	50	20	65	92.5	85	90	97.5
Arran Banner	2.5	2.5	0	7.5	7.5	12.5	15.5
King Edward	35	32.5	75	55	67.5	57.5	90
Dunbar Standard	65	57.5	95	92.5	100	100	95
Golden Wonder	17.5	22.5	32.5	32.5	82.5	72.5	87.5
Mean	31	30	47	56	65	68	87

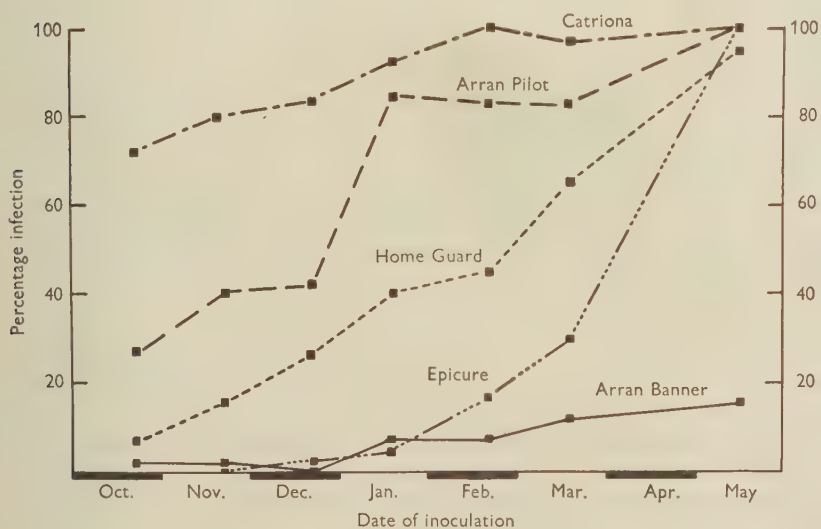


Fig. 1. The percentage dry rot developing in five potato varieties inoculated at monthly intervals in 1949-50.

series are shown in Table 1 and in part again in Fig. 1. All varieties, whether susceptible or resistant, show a similar trend and in general the increase in susceptibility proceeds at a fairly uniform rate until early summer when it is 100 % in all

but the very highly resistant varieties. Fluctuations occur, particularly in the variety Majestic, but they do not affect the main trend even in this variety. The detailed reactions of individual varieties, however, will be discussed more fully in a later paper.

(2) *Variation in immature tubers.* Experiments by R. J. Scott of the Department of Agriculture for Scotland were started in 1938 to determine susceptibility of tubers lifted before maturity. This work was continued by C. E. Foister in 1942-4, and samples of Arran Pilot and Doon Star tubers were lifted at several centres in Scotland at weekly intervals from late July till early October, sent by rail to East Craigs where they were riddled immediately and stored in boxes until the following March. Counts of the amount of dry rot developing in the different samples indicated that there was a peak of susceptibility reaching in some cases as much as 80 % in early or mid-August. A second but rather lower peak occurred in late September or early October. The dry rot occurring in these experiments was the result of infection of wounds caused either in lifting, in transport or in riddling, by field soil which carried the fungus. Although factors such as soil infestation and transport damage are variable, the very high maximum of susceptibility obtained was much too consistent to be fortuitous.

It was therefore decided to repeat this work keeping the variable factors to a minimum. Tuber samples of $\frac{1}{2}$ cwt. each were taken by hand from twelve commercial crops of Arran Pilot in Fife, Mid- and East Lothian in 1948 at weekly intervals from the end of July until early October when the crops were lifted. Monthly sampling from the clamps was continued until spring, care being taken to select undamaged tubers of seed size. The samples were placed in sterilized bags brought carefully by road to East Craigs and riddled the following day for a standard time on a previously sterilized hand riddle. They were then returned for 3 weeks to the bags, where the more humid environment would favour infection, and finally placed in sprouting boxes until spring. The final dry-rot counts, however, revealed only a very low incidence of the disease in practically every case and there was no clear evidence of any pre-lifting susceptibility peak.

The experiment was repeated in 1949 by sampling tubers from six fields in a small area in East Fife carrying commercial crops of Doon Star. The samples, of about $\frac{1}{3}$ cwt. each, were treated as in the previous experiment. The final dry-rot counts, however, again showed that the general level of infection was extremely low but one sample showed a susceptibility peak of 21 % in mid-August, and another a peak of 15 % at the end of August.

Neither of these experiments provides much evidence for the extremely high susceptibility peak in immature tubers observed in Foister's work. However, in 1949, samples of twenty Doon Star tubers from each of the six fields were taken from the larger samples prior to riddling, washed, surface sterilized and inoculated by the standard technique in the laboratory 2 days later, except sample 7 which was inoculated 9 days later. Sampling of the crops was carried out at 7-16-day intervals

from the time of flowering, at the beginning of August, until lifting in early October and thereafter from the clamps at monthly intervals until spring. Table 2 gives the general state of the haulm and tubers at the sampling dates up to lifting time while the results of the inoculations are shown in Table 3 and Fig. 2.

TABLE 2. *Condition of haulm and tubers of Doon Star plants at sampling dates in 1949*

Date of sampling	State of haulm	State of tuber
9 August	Flowering just declining	Few ware size: skinned on riddling
16 August	Completely green and vigorous	Skinned on riddling
23 August	Green: trace of blight	Skinned on riddling
31 August	Blight 1-75 %	Skinned on riddling
12 September	Most leaves dead: (source A— haulm killed by H ₂ SO ₄)	Skinned on riddling (except source A)
26 September	Completely dead	Not skinned on riddling
12 October	Completely dead	All samples taken from clamp (except sources C and F)

TABLE 3. *Percentage dry rot in samples of Doon Star tubers from six sources (A-F) inoculated at intervals from August 1949 until March 1950*

Sample no.	Date	A	B	C	D	E	F	Mean
1	9 Aug.	17.5	17.5	30	32.5	10	5	18.8
2	16 Aug.	75	37.5	30	77.5	57.5	25	50.4
3	23 Aug.	65	65	32.5	85	52.5	65	60.8
4	31 Aug.	77.5	42.5	12.5	50	60	50	48.8
5	12 Sept.	2.5	0	0	0	0	0	0.4
6	26 Sept.	0	0	5	0	10	0	2.5
7	12 Oct.	7.5	20	12.5	15	17.5	10	13.8
8	21 Nov.	17.5	0	5	10	2.5	0	5.8
9	15 Dec.	52.5	27.5	7.5	12.5	2.5	0	17.1
10	17 Jan.	47.5	7.5	0	40	35	2.5	22.1
11	21 Feb.	35	52.5	45	55	27.5	—	43.0
12	15 Mar.	50	42.5	—	72.5	32.5	—	49.4
Mean (omitting 11 and 12)		36.3	21.8	13.5	32.3	24.8	14.5	

Tables 2 and 3 and Fig. 2 show that a peak of susceptibility occurs towards the end of August at a time when the haulm, although green, appears to be losing vigour. This would correspond approximately to the beginning of the maturity stage as described by Singh & Mathur (1937). There is also a tendency for a small peak to occur early in October, but since most of the samples at this time were taken from tubers already lifted and in clamps where storage conditions may have varied and since they were inoculated 9 days instead of 2 days after sampling other factors may have been introduced. However this small peak is also shown by tubers from sources C and F which were taken straight from the soil. After this second peak, the mean figures follow the pattern of the previous seasonal susceptibility experiments and increase gradually until spring.

The low result on 12 September is hardly likely to have been due to the 8 % germination of the spore inoculum on that date in view of the fact that a similar low infection resulted at the following inoculation date when the activity of the suspension was normal, i.e. 92 %.

Pre-lifting susceptibility was also noted in 1950 after periodic inoculation of immature tubers of the variety Catriona, and a maximum of 52.5 % was reached again on 23 August. Very little infection, however, was obtained in similar inoculations on tubers of May Queen and Ninetyfold and none at all with Arran Banner. It may have been that the susceptibility period had been passed in the two early varieties when sampling was commenced on 8 August and there may be none in the resistant main crop variety Arran Banner.

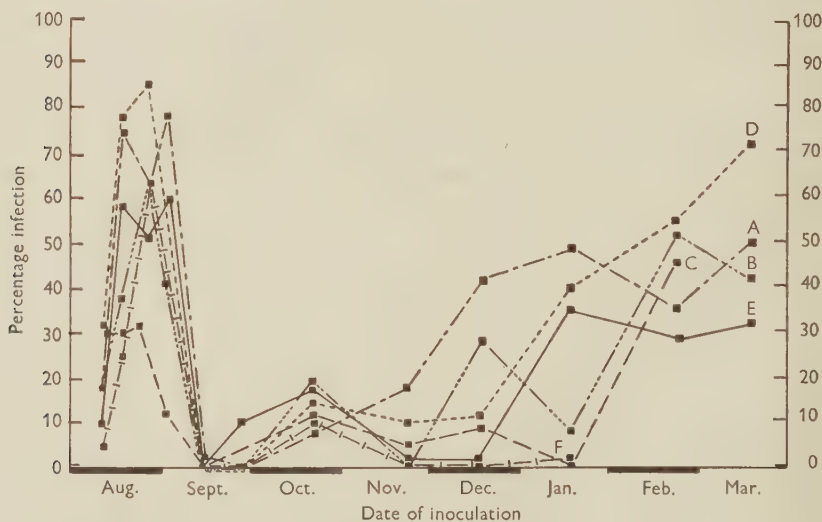


Fig. 2. Percentage dry rot in Doon Star tubers from six sources (A-F) inoculated at intervals from August 1949 until March 1950.

Samples of tubers taken from the field and the clamp at different dates and subsequently stored in boxes were inoculated in the following May, but few differences were observed and even those samples which had originally shown resistance were by that time practically 100 % susceptible.

(3) *Variation with source.* Table 3 also provides some indications of variations between stocks from different sources. The mean figure for each source is shown but the results of the last two inoculations were omitted in arriving at these figures since no more tubers were available at source F after January nor at source C after February. Table 3 reveals wide variations between individual samples from different sources lifted on the same day, e.g. 77.5 % (source A) and 12.5 %

(source C) on 31 August. This might be attributed to the crops not being exactly at the same stage of growth, but differences are still obvious in samples taken from the clamps, although here again some difference in storage conditions may have been responsible. However, the orders of susceptibility of the different stocks before commercial lifting and between October and January differ only in that the orders of the two highest, A and D, and of the two lowest, C and F, have been reversed.

Each of the six crops was given approximately the same amounts of farmyard manure (10–15 tons per acre) and complete potato fertilizer, and the seed planted at about the same time, mid-April. All the soils were light or heavy medium loams except soil D, which was a sandy loam, but neither soil type nor acidity, which varied from pH 5.5 to 6.8, appeared to affect dry-rot susceptibility. The available potash and phosphate was medium to high in all fields in this experiment, and also in 1950 when it was repeated on other fields on a smaller scale but with similar results. Here also the loss on ignition and sticky-point measurements gave no guide as to the cause of the differences.

In order to obviate differences in cultural and soil conditions and to find if variations in reaction to *F. caeruleum* occurred in tubers grown in the same soil from seed of different origins, similarly sized samples taken from the six sources in the 1949 experiment, were planted in a 6 × 6 Latin square in 1950, fifteen tubers in each plot. No differences were observed in rate of emergence. The plots were sampled at weekly intervals from 8 August, when flowering was just over, until 26 September when the haulms were dead. Similarly sized tubers were taken from one plant in each plot and the tubers from the six plots of each stock combined at each sampling. The remaining tubers were all lifted in October and the produce of the six stocks stored separately. A final inoculation was carried out in January. It was found impracticable to carry out inoculation of samples from each of the thirty-six separate plots at each sampling date, and consequently a statistical analysis of the results in Table 4 is not possible.

TABLE 4. *Percentage dry rot in Doon Star tubers from six original sources grown on the same land and sampled and inoculated at intervals from August 1950 to January 1951*

Sample no.	Date	A	B	C	D	E	F	Mean
1	8 Aug.	17.5	40	5	15	15	27.5	18.3
2	17 Aug.	2.5	5	2.5	0	5	0	2.5
3	23 Aug.	30	7.5	20	25	37.5	10	21.7
4	30 Aug.	7.5	2.5	22.5	10	12.5	15	11.7
5	6 Sept.	12.5	10	2.5	12.5	5	2.5	7.5
6	13 Sept.	2.5	12.5	0	7.5	0	2.5	4.2
7	20 Sept.	0	2.5	0	0	0	0	0.4
8	26 Sept.	0	0	0	2.5	0	0	0.4
9	26 Jan.	72.5	67.5	55	57.5	75	82.5	68.3
	Mean	17.2	17.6	13.6	15.3	17.8	16.5	

These results show again the susceptibility maximum at the same date, 23 August, as in 1949, but the general level of infection in the pre-harvest samples was much lower. The maxima in stocks B and F, however, appear on 8 August, but the reason for this and the very low infection on 17 August is not known. Wide variations are also present between the stocks at given inoculation dates. The differences, however, in the means for the six stocks are remarkably small and obviously not significant, but it is curious that stock C again shows the lowest susceptibility. Although more detailed work would be necessary for confirmation, it seems likely that any differences in susceptibility between separate stocks of one variety arise from different conditions of growth and are therefore not genetical. It appears essential also to grow tubers used in any inoculation experiments for comparative purposes under the same conditions and in the same soil.

DISCUSSION

Seasonal susceptibility of tubers to *F. caeruleum* has again been demonstrated in several potato varieties. The increase was gradual from the time of normal lifting in October until the following spring when even relatively resistant varieties became very susceptible. An outstanding exception was Arran Banner which remained highly resistant even in May. Each variety had its own rate of susceptibility increase. The late-season diminution in inter-varietal differences indicates that testing should be done not later than February. Preferably tests should be made both in November and in February, to determine loss of resistance during storage.

Pre-harvest susceptibility to *F. caeruleum* has now been confirmed by laboratory inoculations, although parallel abrasion inoculations with field soils resulted in little dry rot. As Arran Pilot and Doon Star are inherently susceptible, as shown by laboratory inoculation, this result must be due to external factors. The riddle was sterilized between treatments and this, together with the fact that usually there was little adherent soil, is the most probable reason. It is not likely that the soils themselves were of low infectivity. It is possible that in practice the bulk of the infective material is not so much in the soil surrounding the tubers but in that adhering to the riddle. The injection inoculations have shown that this pre-harvest susceptibility is not caused by lack of mechanical resistance of the immature skin but to physiological changes in the developing tuber.

Changes in composition in immature tubers occur constantly. According to Singh & Mathur (1937) and Appleman & Miller (1926) rapidly growing tubers in the post-flowering period show a high sucrose/hexose ratio which falls sharply when the leaves start to die. Pethybridge & Lafferty (1917) discounted the action of sugar concentration in influencing infection since they could not increase susceptibility by low-temperature storage before inoculation. Moore (1924) also states that differences in susceptibility do not appear to be due to variations in sugar content. Pre-maturity susceptibility probably depends on the presence of

a transitory compound or compounds which are concentrated during active growth of the tubers and which are then converted to more stable substances as the potatoes mature and become dormant.

As tubers break dormancy or as external storage factors change, these compounds may be reformed and susceptibility of a stock will depend on the rate at which this occurs in the individual tubers.

Small (1944, 1945) found that inoculation of freshly dug Ninetyfold tubers in July or August caused dry rot to develop quickly in some seasons only. In much of Small's work the crop had been harvested immature and the failure of his inoculations in some seasons may have been due to the changes in pre-maturity susceptibility already described.

Differences also exist in the susceptibility of different stocks of the same variety of potato. Such differences may, as Lansade (1949) suggested, be due to differences in soil. Mooi (1950) found that potatoes grown in heavy soil are often more resistant than those grown on sandy soils, but consistent evidence for this or for the effects of other physical and chemical soil factors upon dry-rot susceptibility were not forthcoming in these experiments. Lansade associated such susceptibility with early sprouting giving sprouts which are longer and thinner than normal. Of the six stocks of Doon Star used in the experiments described in this paper stock C showed some sprouting in the clamp in January but no dry rot developed on inoculation, whereas in the other stocks in which no sprouting occurred at that time, dry rot developed to a moderate extent. No clear association was observed between the appearance of the tubers when taken from the clamps and their reaction to *F. caeruleum*. The over-all level of infection in the pre-harvest samples in the 1950 inoculations was much lower than that in 1949, and it is possible that this may have been associated with generally wet and dry growing seasons respectively.

There was no evidence that differences in susceptibility between different stocks are transmitted to the succeeding crop. Variations at any given date between comparable samples were often large, suggesting slight differences in maturity rates between individual plants, since a considerable fluctuation in susceptibility can occur even in a few days.

The author is very grateful for the co-operation of many seed potato growers in the East of Scotland.

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DRY-ROT DISEASE OF THE POTATO

VI. VARIETAL DIFFERENCES IN TUBER SUSCEPTIBILITY OBTAINED BY INJECTION AND RIDDLE-ABRASION METHODS

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(With 1 Text-figure)

By combining the results of the standard laboratory injection test and a riddle-abrasion method of inoculation, both carried out at different periods of the year, a susceptibility index for a number of commercial potato varieties grown on the same soil was obtained, using the variety *Catriona* as a standard. None of the varieties tested was immune from the disease.

The reactions of certain varieties were found to differ in both tests and in a few, e.g. *Golden Wonder*, this is believed to be caused by differences between 'physiological' susceptibility, i.e. that of the tuber flesh, and 'mechanical' susceptibility, i.e. where liability to abrasion is also involved.

A method for assessing skin abrasion is described.

The rate of development of rotting was found in some cases to be independent of the final susceptibility index.

Among the first to recognize differences in susceptibility of potato varieties to *Fusarium caeruleum* were Pethybridge & Bowers (1908) who recorded heavy losses in the variety *Snowdrop* in Ireland. In 1917, Pethybridge & Lafferty inoculated tubers of several varieties in the laboratory and found that *Epicure*, *Windsor Castle* and *Eclipse* were less susceptible than *Ninetyfold*, *Early Puritan*, *Sharpe's Express* and *May Queen*.

Foister (1940) found that the early and second early varieties *May Queen*, *Ninetyfold*, *Sharpe's Express*, *Duke of York*, *Catriona* and *Di Vernon* were frequently very severely attacked, while *Dargill Early*, *Arran Pilot*, *Arran Comrade* and *Majestic* sometimes sustained serious losses. Moore (1945) found decreasing susceptibility in the series *Doon Star*, *Majestic*, *King Edward* and *Arran Pilot* when the initial temperature of storage was 15° C. At lower initial temperatures the position of the last two varieties was reversed.

In France, Bretignière (1949) noted that *Doon Star* and *Record* were more susceptible than *Bintje* and that *Saucisse*, *Rosa* and *Primula* were resistant. Lansade (1949) listed continental varieties assessed for susceptibility in the laboratory in comparison with *Bintje*.

In Great Britain, early varieties have been found generally more susceptible than late varieties. The early main crop variety *Doon Star* has also shown very heavy

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losses. Fig. 1 shows the acreage of Doon Star in Scotland grown for seed and inspected by the Department of Agriculture since 1933. In 1943 with 12,046 acres (10.1 % of the total inspected acreage) Doon Star was second only to Majestic (28.8 %) but by 1950 Doon Star was 14th with 1120 acres (1.08 %), while Majestic (34.6 %) continued first. A small increase occurred in 1951, but the general decrease in the popularity of Doon Star can be attributed both directly and indirectly to the effect of dry rot.

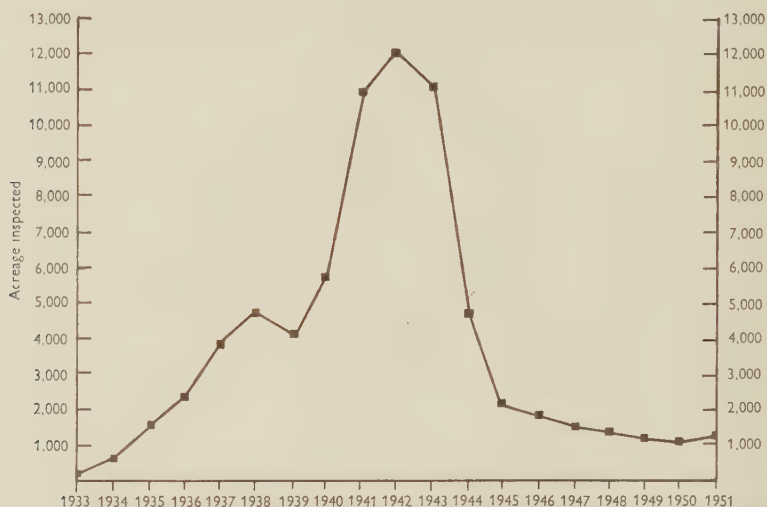


Fig. 1. The acreage of the variety Doon Star officially inspected in Scotland, 1933-51.

MATERIALS AND METHODS

(1) *Laboratory injection method.* The method used was that described by Boyd (1952a), all the tubers used in each experiment being grown in the same field. Many varieties were tested periodically over several seasons from 1947 to 1950, and in several tests the size of each lesion was measured. In all tests the susceptible variety Catriona was used as a standard.

(2) *Riddle-abrasion method.* Boyd (1947) and Foister & Wilson (1950) showed that in practice most of the dry rot develops after riddling injury. A method of assessing varietal susceptibility was therefore devised by simulating this injury under relatively controlled conditions.

Tubers were surface-sterilized in 2 % formalin on lifting, dried and kept in large new paper sacks in a potato store until required. The inoculum was made up of 5 lb. of a sterilized light loam soil mixed with 500 ml. of a spore suspension of *F. caeruleum* containing approximately 300,000 spores per ml. In each test, fifty tubers per variety were rubbed in this inoculated soil so that each tuber became coated with a film of the wet soil. They were then shaken 50 times on a previously

sterilized 1 in. mesh hand riddle and kept in small sterilized hessian sacks for 3 weeks in the potato store. Subsequently, the bags were carefully emptied into the small, previously sterilized, sprouting trays in which the tubers were finally stored. Throughout the storage period the tubers were kept free from frost. The initial bag storage provided higher humidity and induced more dry rot than when the tubers were boxed immediately after riddling. The potatoes were examined periodically, final counts being taken usually in April. The temperature of the store varied with the external temperature. Tests were carried out at the same time as corresponding laboratory inoculations and many of the varieties were tested at intervals during several seasons.

EXPERIMENTAL RESULTS

(1) *Laboratory method.* In the 1947-8 season twelve commercial varieties were inoculated at 2-monthly intervals. As well as counts of the numbers of positive inoculations, measurements in millimetres were made of the depth and maximum superficial diameter of each lesion. By assuming that the rotted tissue in each lesion took the form of a hemisphere, its volume could be calculated, but for comparative purposes a reasonable size index of the lesions may be obtained from the mean radius. These figures for each variety are shown together with the other results of these tests in Table 1.

TABLE 1. *Percentage dry rot developing and index of lesion size after injection inoculations of twelve varieties at different dates in season 1947-8*

Variety	5 Sept.		11 Nov.		16 Jan.		16 Mar.		Mean		Susceptibility index*
	Dry rot (%)	Size index	Dry rot (%)	Size index	Dry rot (%)	Size index	Dry rot (%)	Size index	Dry rot (%)	Size index	
Catriona	77.5	16.6	97.5	—	100	18.8	97.5	20.3	93	18.6	100
Sharpe's Express	52.5	19.8	100	—	100	17.6	100	16.2	88	17.9	95
Doon Star	85	19.6	82.5	—	100	16.0	62.5	19.3	83	18.3	89
Ninetyfold	32.5	19.3	97.5	—	100	18.2	97.5	20.2	82	19.2	88
Golden Wonder	85	18.6	55	—	70	14.2	57.5	16.2	67	16.3	72
Arran Pilot	15	14.8	50	—	95	17.5	73.5	16.5	58	16.3	62
King Edward	50	20.5	42.5	—	82.5	15.6	57.5	16.9	58	17.7	62
Majestic	30	18.5	27.5	—	100	19.3	70.0	19.1	57	19.0	61
Epicure	20	19.7	32.5	—	55	15.0	45	19.7	38	18.1	41
Redskin	17.5	17.1	20	—	67.5	12.3	45	18.9	38	16.1	41
Kerr's Pink	5	16.7	15	—	72.5	13.1	12.5	14.3	29	14.7	31
Arran Consul	5	16.3	12.5	—	47.5	14.4	32.5	14.8	24	15.2	26

* Regarding Catriona as 100.

Table 1 shows some variations in individual tests, and there is a general fall in the rotting produced by the March inoculations. The very high and very low results for Golden Wonder and Arran Pilot, respectively, in September also appear to be aberrant. The mean results, however, separate the varieties according to

susceptibility into an order reasonably close to that given by field observations. The exception shown by Golden Wonder will be discussed later.

The differences given by the indices of the volume of rotted tissue do not correspond consistently with the numbers of positive inoculations for each variety, but it is quite possible that the rate of rotting may be independent of the number of tubers in a susceptible condition. The varieties Redskin, Kerr's Pink and Arran Consul show the least number of lesions, which are correspondingly small, and there is a general resemblance to the order expected from field observations. The chief exceptions are Majestic, Epicure and Arran Pilot, but the last mentioned appears to be out of position by both methods because of an inexplicably low count on the first inoculation. The rate of progress of the fungus has been shown to vary within the tissue of different varieties (Moore, 1945), and this is the basis of the susceptibility assessment method used by Lansade (1949). Table 1, however, suggests that results more easily obtained and more readily assessed by reason of wider differences are given by the percentage of positive inoculations than by rate of rotting, a conclusion which was confirmed by similar experiments during 1949-50.

TABLE 2. *Percentage dry rot developing in twenty-two varieties after injection inoculation at intervals in season 1948-9*

Variety	25 Oct.	8 Dec.	4 Feb.	8 Apr.	Mean	Susceptibility index
Dunbar Standard	82.5	87.5	57.5	100	82	119
Catrina	50	80	45	100	69	100
Ninetyfold	25	77.5	27.5	100	56	80
Eclipse	35	57.5	20	100	53	77
Arran Pilot	47.5	30	30	100	52	75
Ulster Chieftain	0	55	47.5	100	51	74
Sharpe's Express	17.5	57.5	25	100	50	72
Doon Star	10	60	27.5	95	48	70
Majestic	15	62.5	25	80	46	67
King Edward	32.5	57.5	20	75	46	67
Gladstone	12.5	45	22.5	85	41	60
Kerr's Pink	10	27.5	20	100	39	57
Red King	12.5	42.5	0	85	35	51
Up-to-Date	5	42.5	7.5	85	35	51
Duke of York	7.5	10	12.5	100	33	48
British Queen	0	12.5	17.5	100	33	48
Redskin	0	27.5	2.5	95	31	45
Epicure	0	5	0	95	25	36
Golden Wonder	0	35	5	57.5	24	35
Great Scot	2.5	15	2.5	72.5	23	33
Arran Peak	0	2.5	2.5	87.5	23	33
Arran Banner	0	0	0	55	14	20

Table 2 gives the results of the laboratory inoculations of twenty-two varieties in the season 1948-9. The figures given in the February test are unusually low in all varieties, possibly because of low germination of the spore suspension used, but the results are still of value for comparison. Most of the varieties show a very high susceptibility in the April test, when many of the finer differences between varieties

disappear. The mean figures for each variety correspond in general with field observations and also with the results in Table 1. Again the most outstanding exception is Golden Wonder. The different reactions of this variety in the two seasons may be explained by the fact that in the 1947-8 season (Table 1) the first inoculation was made on 5 September when the tubers of this late variety were immature, and probably in the relatively brief period of high pre-maturity susceptibility shown by Boyd (1952*b*). Dunbar Standard is not normally considered very susceptible in practice although it has been described (Anon., 1940) as 'rather susceptible'. In these tests, however, it showed consistently more infection than Catriona. King Edward is another variety which in practice appears relatively free from dry rot yet the tests in both seasons rate it as susceptible as Majestic.

Further results with nine varieties in the season 1949-50 (Table 1, Boyd, 1952*b*) confirm the position of Dunbar Standard and King Edward. An additional series of nineteen less widely grown varieties was tested twice in season 1949-50 and the mean results are included in Table 6.

(2) *Riddle-abrasion method*. The development of dry rot on riddle abrasions as shown in Tables 3-8 again shows differences between varieties, but these are not always correlated with the variations obtained in the corresponding laboratory tests. In Table 3 the susceptibility order approximates to that of the corresponding laboratory test (Table 1) with a few exceptions. Ninetyfold shows slightly more infection than Catriona but it is doubtful whether this difference is significant; Arran Pilot is nearer to Catriona in the abrasion test than in the laboratory test; while Epicure shows an unexpectedly high figure in Table 3. Golden Wonder takes the lowest place in the abrasion test, possibly because it has a very thick skin which might provide some mechanical resistance to riddle abrasions. This would not be shown by the laboratory test where the skin is broken in each inoculation. Resistance in this variety at least may thus be of two types: 'mechanical' resistance to abrasion and 'physiological' or inherent resistance of the tissue to the fungus. In the riddle-abrasion inoculations both types would come into play.

TABLE 3. *Percentage development of dry rot in eleven varieties after riddle-abrasion inoculation at intervals in season 1947-8*

Variety	5 Sept.	11 Nov.	16 Jan.	16 Mar.	Mean	Susceptibility index
Ninetyfold	12	92	98	100	76	106
Catriona	12	92	100	84	72	100
Sharpe's Express	14	78	96	86	69	96
Arran Pilot	6	68	100	94	67	93
Doon Star	6	70	92	96	66	92
Majestic	0	60	96	68	56	78
Epicure	8	72	82	32	49	68
King Edward	6	36	76	68	47	65
Kerr's Pink	4	32	96	8	35	49
Redskin	0	14	64	42	30	42
Golden Wonder	2	18	56	16	23	32

The results in Tables 4 and 5 which were obtained in 1948-9 and 1949-50, respectively, generally agree with the corresponding laboratory tests, the exceptions in Table 4 being the unexpectedly low abrasion ratings of Ninetyfold, Eclipse and Duke of York. The two latter varieties, however, continued to exhibit a similar

TABLE 4. *Percentage development of dry rot in twenty-one varieties after riddle-abrasion inoculation at intervals in season 1948-9*

Variety	26 Oct.	9 Dec.	5 Feb.	12 Apr.	Mean	Susceptibility index
Catriona	88	100	94	98	95	100
Dunbar Standard	94	98	88	60	85	89
Sharpe's Express	50	100	100	88	82	86
Arran Pilot	46	98	92	92	82	86
Doon Star	88	90	84	44	77	81
Ninetyfold	52	84	—	—	65	68
Ulster Chieftain	72	64	62	48	62	65
King Edward	70	66	48	48	58	61
Gladstone	66	76	12	50	51	54
Majestic	74	58	36	32	50	53
British Queen	4	48	34	30	49	52
Eclipse	12	62	54	62	48	50
Redskin	94	14	44	16	42	44
Kerr's Pink	38	76	30	14	40	42
Up-to-Date	34	60	36	28	40	42
Epicure	6	24	20	48	32	34
Duke of York	8	66	10	28	28	29
Golden Wonder	38	50	4	10	26	27
Arran Peak	32	18	20	8	20	21
Great Scot	14	14	14	4	12	13
Arran Banner	6	20	6	10	11	12

TABLE 5. *Percentage development of dry rot in nine varieties after riddle-abrasion inoculation at intervals in season 1949-50*

Variety	27 Oct.	24 Nov.	19 Dec.	19 Jan.	27 Feb.	20 Mar.	Mean	Susceptibility index
Catriona	68	96	54	72	98	94	80.3	100
Arran Pilot	74	96	74	52	94	86	79.3	99
Dunbar Standard	42	72	38	24	64	76	52.7	66
King Edward	14	48	40	8	68	60	53.0	66
Majestic	8	12	4	10	56	36	21.0	26
Home Guard	12	38	14	2	28	10	17.3	22
Epicure	10	18	4	4	24	26	14.3	18
Arran Banner	4	40	4	10	4	0	10.3	13
Golden Wonder	0	12	6	6	10	10	7.3	9

degree of resistance when tested again by the abrasion method with a series of less widely grown varieties in February 1950. Golden Wonder again shows a high resistance to the riddle inoculation, but in this instance the corresponding laboratory results were also low (Table 2). In the 1949-50 tests this variety again showed a very low abrasion susceptibility (Table 5), coupled with a relatively much higher degree

of 'physiological' susceptibility as shown by the laboratory test (Table 1, Boyd, 1952*b*). Table 5 also shows that Dunbar Standard, which in corresponding laboratory tests was almost as susceptible as Catriona, has a higher degree of mechanical resistance. On the other hand, Arran Pilot is again correspondingly more susceptible in the riddle test. The degree of resistance shown by Majestic in Table 5 is very unexpected in view of its behaviour when inoculated in the laboratory.

In February 1950, a further series of varieties was subjected once to the riddle-abrasion test in conjunction with laboratory injection tests. These results, together with those of all injection and abrasion inoculations between 1947 and 1950, are included in the mean figures in Table 6 which are expressed as percentages of the standard variety Catriona.

The overall susceptibility index given in Table 6 obtained from the mean laboratory and riddle reactions is reasonably close to expectations from field observation. No commercial variety has been found to be immune, but the four varieties, Arran Victory, Arran Banner, Great Scot and Arran Chief are highly resistant, probably the most resistant of all the more widely grown varieties in Great Britain.

Any division which is made in this list is necessarily subjective and arbitrary but a separation at 50 % in the overall susceptibility index is suggested as a dividing line between 'susceptible' and 'less susceptible' varieties. Within the 'susceptible' group, Catriona, Dunbar Standard, Sharpe's Express, Ninetyfold, Doon Star and Arran Pilot could be regarded as 'highly susceptible'. Results of a single abrasion test give some evidence for the inclusion of Orion in this category. Additional data obtained mainly from one injection test in February 1951 of several other varieties gave the following indications:

Highly resistant: Arran Luxury, Conference, Stormont Dawn, Stormont Star, Ulster Earl, Arran Comrade.

Less susceptible: Ulster Ensign, Ulster Cromlech, Ulster Premier.

Susceptible: Ulster Prince, May Queen.

It has been noted previously that the resistance of some varieties shown in the abrasion test may differ from their 'laboratory' or 'physiological' resistance. This is brought out in Table 7 where those varieties are listed which showed differences of more than 10 % and which were subjected to more than one abrasion test.

The category showing lower abrasion susceptibility includes many more varieties than that showing lower injection susceptibility. It was thought that these differences were due to differences in liability to skin abrasion which in the first category was not great but which in the second was high and would lead to an increased chance of infection whatever the inherent susceptibility of the variety.

Tubers of a number of varieties, all grown on the same land and stored under the same conditions until February 1952, were therefore subjected to an abrasion damage test using a metal rod 23 cm. long, 4 mm. in diameter and weighing

TABLE 6. *Dry-rot susceptibility index of varieties tested by injection and abrasion methods. Mean figures for all experiments, 1947-50*

	Injection	Abrasion	Overall	
Arran Victory	16	4*	10	Highly resistant
Arran Banner	13	13	13	
Great Scot	20	13	17	
Arran Chief	19	22*	21	
Arran Consul	26	—	—	Less susceptible
Craigs Royal	38	14*	26	
Arran Peak	33	21	27	
Craigs Bounty	19	37*	28	
Home Guard	39	22	31	
Duke of York	44	19	32	
Up-to-Date	52	24	33	
Epicure	28	40	34	
Dr McIntosh	56	14*	35	
Arran Crest	44	28*	36	
Golden Wonder	52	23	38	Susceptible
Kerr's Pink	43	39	41	
Red King	51	33	42	
Redskin	43	43	43	
Dunbar Rover	56	41*	49	
Craigs Defiance	58	39*	49	
British Queen	48	52	50	
Ballydoon	54	51*	53	
Eclipse	75	34	55	
Gladstone	60	54	57	Very susceptible
Majestic	63	52	58	
Craigs Snow-White	56	59*	58	
King Edward	63	64	64	
Record	58	78*	68	
Ulster Chieftain	74	65	70	
Craigs Alliance	47	92*	70	
Vanguard	68	71*	70	
Orion	60	98*	79	
Arran Pilot	67	93	80	
Doon Star	80	87	84	
Ninetyfold	84	87	86	
Sharpe's Express	84	91	88	
Dunbar Standard	108	76	92	
Catriona	100	100	100	

* One test only, February 1950.

TABLE 7. *Varieties showing differences of more than 10 % between abrasion and injection susceptibility to dry rot*

Abrasion susceptibility less (%)		Injection susceptibility less (%)	
Majestic	11	Epicure	12
Arran Peak	12	Arran Pilot	26
Home Guard	17		
Red King	18		
Duke of York	25		
Up-to-Date	28		
Golden Wonder	29		
Dunbar Standard	32		
Eclipse	41		

23.45 g. with a 4 mm. ball-bearing soldered to the end. This rod was allowed to fall through a measured distance in a glass tube on to the tubers under test. The distance of fall in centimetres which just failed to produce an abrasion was measured, the abrasion being counted when moisture was observed at the site. In each test three blows were made on each tuber and six tubers used for each variety. In all, four tests were made with two sets of tubers, one set in tests 1 and 2 and the other in tests 3 and 4. An interval of 10 days elapsed between tests 1 and 2 and 1 day between tests 3 and 4. The results in Table 8, given as the mean sum of the measurements of the three blows per tuber, suggest that Golden Wonder with the highest overall mean is the most resistant to damage. An analysis of variance, however, revealed a significant interaction between tests and varieties, and thus a standard error based on this variance has been adopted. This is somewhat higher than the normal error and on this basis, differences between means for Golden Wonder, Home Guard and Dunbar Standard are not significant. Golden Wonder, however, is significantly different from all the other varieties. Of those mentioned in Table 7 which were tested, the difference between Dunbar Standard and Arran Peak is not significant nor are those between the latter and the other five varieties. It would appear, therefore, that the differences between abrasion and injection susceptibility to dry rot as shown in Table 7 for varieties such as Eclipse, Duke of York or Arran Pilot are probably not due to any differences in liability to skin abrasion, but the true cause is unknown. On the other hand, resistance to damage may have some effect in varieties such as Golden Wonder and Dunbar Standard, but in most varieties it does not seem to be a major factor in commercial susceptibility to dry rot.

TABLE 8. *Susceptibility to damage of eighteen potato varieties as measured by mean sum of three blows per tuber, six tubers per variety per test (February 1952)*

Variety	Test 1	Test 2	Test 3	Test 4	Mean
Golden Wonder	27.0	26.9	32.3	26.3	28.1
Home Guard	21.2	29.3	26.2	24.0	25.1
Dunbar Standard	19.8	19.8	28.2	26.5	23.6
Arran Victory	21.3	26.2	22.7	21.0	22.8
Record	23.5	21.5	21.0	23.2	22.3
Orion	21.7	21.7	21.5	22.7	21.9
Arran Peak	22.7	24.0	13.2	19.3	19.8
Dr McIntosh	17.2	17.2	22.0	16.2	18.1
Craigs Royal	16.5	17.2	18.7	19.7	18.0
Majestic	12.7	18.3	21.3	18.7	17.8
Arran Crest	19.2	18.8	14.5	18.5	17.8
Arran Pilot	16.5	18.3	16.2	18.7	17.4
Epicure	17.5	11.5	21.0	19.0	17.3
Craigs Alliance	18.3	15.0	16.8	18.7	17.2
Eclipse	18.2	14.7	14.5	17.2	16.1
Dunbar Rover	18.7	20.5	10.3	13.5	15.8
Duke of York	11.8	19.0	14.7	15.2	15.2
Craigs Defiance	13.8	10.5	15.7	17.2	14.3
Difference necessary for significance ($P=0.05$)	2.96	3.39	4.79	2.00	4.93

Rate of dry-rot development

In some riddling tests counts of tubers showing dry rot were made at intervals up to mid-July, care being taken that none of the tubers were damaged. Table 9 gives an indication of varietal differences in rates of development of the disease. The tubers were riddled by the standard method on 27 February 1950, and counts made on 12 May, 16 June and 13 July.

TABLE 9. *Rate of development of dry rot in different varieties, after abrasion test (February 1950)*

Variety	Percentage dry rot developing after		
	74 days	109 days	136 days
Arran Pilot	94	94	100
Catriona	96	98	98
Craigs Alliance	78	90	96
Craigs Royal	4	14	88
Record	20	76	86
Craigs Snow-White	10	58	84
Vanguard	60	70	80
Kerr's Pink	10	26	76
Epicure	8	24	74
Craigs Bounty	26	36	66
Ballydoon	30	50	56

In the very susceptible varieties, e.g. Catriona, the progress was rapid, but even in the initially more resistant varieties, e.g. Craigs Royal, the final counts in July were very high. Other varieties such as Craigs Bounty and Ballydoon did not show such rapid development after moderately high initial dry-rot counts.

It is known that by July practically all varieties of potato are very susceptible to the disease. In injection inoculations, although some tendency towards differential progress of rotting was observed, as indicated in Table 1, practically all the dry rot had become obvious after 6 weeks' incubation at 60° F. and about 94 % relative humidity. After riddling, however, the tubers were kept in open boxes in a potato store after an initial 3-week period of bag storage. The temperature was uncontrolled and was relatively low (41–54° F.), but the humidity was fairly high (85–95 % R.H.) especially while the tubers were in bags.

DISCUSSION

The results of the experiments which have been described show that the standard laboratory injection test alone, of which extensive results using many varieties are discussed in the text, can demonstrate reasonably fine differences in susceptibility. It takes no account, however, of any possible effect which might be produced by the resistance of the outer tissues of the tuber to abrasion, since it has been shown that in commercial practice small skin abrasions such as those produced on the mechanical riddle are responsible in most cases for the entry of the dry-rot fungus.

The standard riddle abrasion test for dry-rot susceptibility which has been described takes this factor into account and simulates what occurs in commercial practice. By carrying out both tests concurrently a more complete picture may be obtained of the susceptibility of a variety. Since conditions of storage in the laboratory test are controlled to a much higher degree the results are believed to be more accurate as well as being more quickly obtained than in the abrasion test. With most of the varieties tested there is a general agreement between the results of each test which suggests that skin resistance is in general not a factor of great importance in dry-rot susceptibility. However, a number of varieties showed wide differences in the results between the tests (Table 7) and in most of these cases susceptibility shown by the abrasion method was lower.

If this is in fact a real effect of skin resistance it should be shown by the standard abrasion assessment technique which was developed for this purpose. By this test certain significant differences in liability to damage were obtained between varieties, and this was quite independent of the susceptibility of the tuber tissues to dry rot. In Golden Wonder, however, and also in Dunbar Standard, varieties whose tissues are susceptible to dry rot, the outer tissues showed a resistance to abrasion significantly greater than most of the other varieties tested. This corresponds with the fact that these two varieties were much more resistant to dry rot in the riddle-abrasion test than in the injection test. There appear therefore to be some varieties where mechanical resistance operates and the concurrent use of both injection and riddle-abrasion methods is advocated.

Out of more than forty commercial varieties tested, none was found to be immune and it is obvious that, in making assessments, reference should be made to an arbitrary standard. The variety Catriona, which is probably the most highly susceptible of all the commercial varieties, appears well suited for this purpose.*

As the final susceptibility of different varieties was not always related to the amount of rotted tissue produced in the injection inoculations, the criterion of lesion size was not adopted. Furthermore, in riddle-abrasion tests the rate of development of dry rot could not in all cases be correlated with the final susceptibility of the varieties. In commercial storage also, some lesions may become obvious only in late spring, 3 or 4 months after wounding and thus presumably after the entry of the fungus. These facts depend on the conditions of storage and make it necessary to carry out the final readings at a fixed time.

Dry rot usually developed relatively rapidly after riddle abrasion in the very susceptible varieties, but some of those where initial development was not high, showed a high disease count after more prolonged storage. If in such cases the fungus is occluded initially by a suberized layer or wound periderm it must be

* It must be remembered that in arriving at the susceptibility list given in Table 6, the varieties were tested under conditions which were as uniform as possible. In practice, there are so many variable factors, e.g. time of riddling, which may affect the amount of dry rot produced that it is frequently unsafe to attempt to assess relative varietal susceptibility.

assumed that this is later broken down as the tuber becomes older and loses turgidity. It has been shown, however (Boyd, 1952*a*) that, in prolonged storage under conditions satisfactory for wound periderm formation, very few arrested lesions became active. It is more likely, therefore, that the fungus enters at the time of riddling and that no protective layer is formed because of unsuitable temperature conditions. The failure of the fungus to spread must then be due to the tuber tissue being not physiologically susceptible. Later, when most varieties show fairly high susceptibility, it is assumed that the tissue changes in constitution, becomes susceptible and the fungus spreads.

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DRY-ROT DISEASE OF THE POTATO

VII. THE EFFECT OF STORAGE TEMPERATURE UPON SUBSEQUENT SUSCEPTIBILITY OF TUBERS

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The standard injection method of inoculation was used in conjunction with the riddle-abrasion method, at three periods during the storage season, to determine the effect of storage of potatoes of three varieties at three temperatures or ranges of temperature upon their subsequent susceptibility to *Fusarium caeruleum*.

Susceptibility was greatly increased by increase in storage temperature. Low-temperature storage (39° F.) before inoculation decreased susceptibility. If, however, such storage immediately followed inoculation, although little or no signs of rotting were obvious after 6 weeks, when these tubers were replaced for a further 6 weeks at the normal temperature of incubation (60° F.) the amount of rotting was enhanced.

Some practical consequences of these facts are discussed.

It is known that tubers become more susceptible to dry rot the longer they are stored under normal conditions (Boyd, 1952*b*). It should be possible therefore by altering the storage temperatures, and thus hastening or retarding the breaking of dormancy of the tuber, to alter its susceptibility.

MATERIALS AND METHODS

About 2-3 cwt. of tubers of the three varieties Arran Pilot, Catriona and Home Guard were surface-sterilized at lifting time in 2 % formalin and then stored in large paper sacks in three ways prior to inoculation: (a) in a laboratory at East Craigs where the normal temperature was about 58° F. with a range of 55° to 78° F. over the period of the experiment; (b) in a thermostatically controlled cold store at a temperature of $39 \pm 1^\circ$ F.; (c) in a potato store where the temperature fluctuated between 34 and 65° F. over the period of the experiment. Separate storage was commenced as soon after lifting as possible, the dates being 8, 21 and 22 October 1948 for (c), (b) and (a) respectively.

The three inoculation treatments were as follows: (1) inoculated by standard injection method; (2) inoculated by riddle-abrasion method; (3) as (1) but incubated thereafter in cold store. Inoculation and incubation methods have been described in previous papers (Boyd, 1952*a, c*). In the laboratory inoculations, twenty (occasionally eighteen) tubers were used. As before, fifty tubers were used for riddle inoculations, final dry-rot counts being made on 27 May 1949. Tubers which had

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been wounded only, without application of the inoculum, were used as controls for treatments (1) and (3). Shortage of tubers did not permit treatment (3) to be applied to tubers given storage treatment (c). In treatment (3) the tubers were incubated on damp peat in cardboard boxes placed in the cold store at 39° F., but when the assessment was made after 6–7 weeks the tubers were kept in the normal incubator at about 60° F. for a further 7–8 weeks.

There were three inoculation dates, 8 December, 4 February and 8 April. In all treatments tubers were desprouted if necessary before inoculation. This was necessary, particularly in April, when tubers stored in the laboratory were used and especially with the varieties Arran Pilot and Home Guard.

EXPERIMENTAL RESULTS

The results of the laboratory inoculations are shown in Tables 1–3. Table 2 shows that the amounts of dry rot are generally lower than the corresponding figures in Table 1 in spite of the fact that the inoculations were made later in the season (February), and this is believed to have been the result of the low germination of the spore suspension used.

TABLE 1. *Percentage dry rot developing in three potato varieties after various methods of storage and treatment. Inoculated 8 December 1948*

Original storage	Treatment	Arran Pilot	Catriona	Home Guard
Laboratory, 58° F.	Inoculated: normal incubation for 7 wk.	72·5	92·5	5
	Inoculated and incubated at 39° F. for 7 wk.	0	0	0
	As above after normal incubation for further 7 wk.	95	97·5	47·5
Cold store, 39° F.	Inoculated: normal incubation for 7 wk.	27·5	67·5	0
	Inoculated and incubated at 39° F. for 7 wk.	0	0	0
	As above after normal incubation for further 7 wk.	70	95	7·5
Potato store, 34–65° F.	Inoculated: normal incubation for 7 wk.	30	80	5

The control tubers showed no dry rot except in the April series (Table 3), which had been stored in the laboratory and, as a result, had become rather flaccid. Control results have therefore been omitted from Tables 1 and 2. Catriona, which is the slowest in sprouting of the three varieties and therefore least flaccid, was by far least affected in this way in April, although it is normally much more susceptible than the other two, but the reason for this unusual extraneous infection is unknown. In practically all inoculations the expected order of varietal susceptibility is demonstrated, i.e. Catriona, Arran Pilot and Home Guard.

Comparing the results given by the standard inoculation method using tubers stored in different ways, it is clear that the higher the temperature of pre-storage the higher the resulting susceptibility of the tuber. This holds for the three types

of storage used in practically every case. Although the temperature ranges in the laboratory (55–78° F.) and in the potato store (34–65° F.) overlap, temperatures were never the same at any one time.

TABLE 2. *Percentage dry rot developing in three potato varieties after various methods of storage and treatment. Inoculated 4 February 1949*

Original storage	Treatment	Arran Pilot	Catriona	Home Guard
Laboratory, 58° F.	Inoculated: incubated normally for 6 wk.	47·5	57·5	27·5
	Inoculated and incubated at 39° F. for 6 wk.	0	0	0
	As above after normal incubation for further 8 wk.	67	61	44
Cold store, 39° F.	Inoculated: incubated normally for 6 wk.	12·5	50	7·5
	Inoculated and incubated at 39° F. for 6 wk.	0	0	0
	As above after normal incubation for further 8 wk.	22	11	11
Potato store, 34–65° F.	Inoculated: incubated normally for 6 wk.	30	45	15

TABLE 3. *Percentage dry rot developing in three potato varieties after various methods of storage and treatment. Inoculated 8 April 1949*

Original storage	Treatment	Arran Pilot	Catriona	Home Guard
Laboratory, 58° F.	Inoculated: incubated normally for 6 wk.	100	100	100
	Wounded only	92·5	5	62·5
	Inoculated and incubated at 39° F. for 6 wk.	47·5	15	27·5
	As above after normal incubation for further 8 wk.	97·5	95	67·5
Cold store, 39° F.	Inoculated: incubated normally for 6 wk.	82·5	100	50
	Wounded only	0	0	0
	Inoculated and incubated at 39° F. for 6 wk.	0	0	0
	As above after normal incubation for further 8 wk.	72·5	100	50
Potato store, 34–65° F.	Inoculated: incubated normally for 6 wk.	100	100	92·5

Irrespective of previous storage temperature, where tubers were inoculated and incubated at 39° F., no infection was obvious after 6–7 weeks, except in those inoculated relatively late in the season, i.e. April or May. However, when such tubers were then transferred to the normal temperature of incubation, i.e. about 60° F. (the relative humidity was probably much the same) fairly rapid rotting took place, indicating that the fungus had remained dormant within the wounds, and that the tubers had not succeeded in forming a protective barrier during the low-temperature storage. Sections through such wounds showed that only a slight suberin layer but no wound periderm had been formed, whereas a substantial cork layer had been produced in the corresponding tubers stored at 60° F. Thus, when the tubers incubated at 39° F. were placed at a more favourable temperature for

both wound periderm formation and for infection, the fungus could attack immediately. The resulting infection, irrespective of the original storage temperatures, was higher in practically every case, except in April when the tubers are naturally extremely susceptible, than that given by a straightforward inoculation followed by immediate normal incubation for 6 weeks. This cannot be the result merely of a different length of incubation period since it has been shown previously that a normal incubation of 6 weeks will reveal the optimum amount of dry rot.

Table 4 shows the results of the inoculations by the riddle-abrasion method.

TABLE 4. *Percentage dry rot developing in three potato varieties previously stored in three different ways, inoculated by riddle abrasion on three different dates and subsequently kept in boxes in potato store. Season 1948-9*

Variety	Original storage	9 December	5 February	12 April
Arran Pilot	Laboratory	80	92	100
	Cold store	82	84	38
	Potato store	98	92	92
Catriona	Laboratory	100	96	100
	Cold store	88	88	84
	Potato store	100	94	98
Home Guard	Laboratory	4	46	100
	Cold store	0	4	0
	Potato store	70	28	40

In the first and second inoculations final dry-rot counts were made on 27 May and in the third they were made on 5 July. No conclusions on seasonal susceptibility can be deduced from this experiment since the suspensions used to inoculate the soil in which the tubers were riddled were not exactly standardized. In the February inoculations, however, where tests showed a low spore germination in the suspension used a fairly high development of dry rot occurred.

The tubers taken from low-temperature storage, even where this was only about 6 weeks as in the earliest inoculation, again show consistently less dry rot than those previously held under the normally fluctuating conditions of a potato store. Those from the warmer laboratory storage were again generally more susceptible. These results tend to support those of the laboratory experiments, but there are two obvious exceptions to be seen in the December inoculation where the result for Arran Pilot stored in the laboratory is rather lower than expected, and more particularly that for Home Guard kept in the potato store, which is unaccountably high. The evidence of all the other results is fairly consistent and suggests that the two aberrant results are due to experimental error.

Tubers, especially those of Arran Pilot, from low-temperature storage inoculated by the abrasion method in April, tend to show slightly more resistance than in the previous two inoculations, even in spite of the lateness of the storage season which would normally enhance susceptibility. Laboratory experiments indicate that where an intermediate period of cold-store incubation preceded normal incubation

a higher infection rate resulted than where normal incubation was used alone. During incubation of the riddle-inoculated tubers, the temperature of the potato store fell to 34° F. during the winter but was generally much higher by April. The first two riddle inoculations therefore had an intermediate period of low-temperature incubation while the April inoculations did not.

In another experiment a commercial stock of Arran Pilot seed was hand picked from a potato clamp into bags on 2 February 1948. After 3 weeks, 2 cwt. of these were stored in boxes in a potato store at East Craigs until 5 June, when 17 % of dry rot had developed and little further development appeared likely. A bag containing $\frac{1}{2}$ cwt. was placed at the same time in a cold store at 39° F., removed on 14 May, and the tubers carefully placed in boxes in the potato store. No dry rot was obvious at this time, but 21 % had developed by 5 June and 41 % by 8 July. Although the two sets are not strictly comparable, it appears probable that the low-temperature storage subsequent to the time of damage, which was in this case removal from clamp and transport to the store, caused an increase in the development of dry rot.

DISCUSSION

The evidence obtained, using the three varieties Arran Pilot, Catriona and Home Guard, indicated that susceptibility of potato tubers to dry rot was increased by increase of storage temperatures even though this period was within 2 months of the lifting date, the conditions of inoculation and incubation being uniform in each case. The lowest temperature used was 39° F., and effected in many cases a considerable reduction in susceptibility as compared with the normal temperature of a potato store over the same periods (34–65° F.). This effect is probably due to the retardation of the normal maturity process within the tuber, and a similar effect has been shown by Mooi (1950) to be caused by the sprout-depressant tetrachloronitrobenzene. These results, however, are at variance with those of Lansade (1949), who found that tubers kept at a low temperature become more susceptible. He found that tubers stored for 13 days at 1–2° C. before inoculation showed an average penetration by the fungus of 21·1 mm., while those stored for similar periods at 10–12 and 20° C., showed penetrations of 10·8 and 4·2 mm. respectively. On the other hand, he indicates that storage in a warm dry place is to be avoided. It is not easy to reconcile these two sets of results, and it may be that the slightly lower temperature and the shorter period of storage in the French experiments is responsible.

Steward (1943) has shown that there is a time lag in the initiation of wound periderm caused by previous storage at a low temperature, e.g. 2° C. This was suggested by McIntosh (1944) as the reason for the increased dry rot observed by him to occur in tubers stored at temperatures below 32° F. after riddling. He also concluded that the increase in sugar content at low temperatures might account for the increase in dry rot.

Tables 1-3 show that low-temperature storage before inoculation even for as little as 6 weeks (Table 1) decreases susceptibility to dry rot, but low-temperature storage subsequent to inoculation, if followed by normal incubation at 60° F., appears to increase it. In McIntosh's experiment the natural wounding took place before the onset of cold weather.

According to Steward (1943), tubers from a cold store should show a lag in wound periderm formation and also presumably in suberization. If such tubers are incubated normally at 60° F. the fungus should be able to penetrate immediately without much mechanical opposition and, therefore, induce enhanced dry rot. But this, according to the present work, is not so. In practically all instances it leads to a lower infection.

Again, incubation for 6 weeks at 39° F. resulted in no obvious infection except in April when tubers usually become very susceptible. When, however, such tubers were subsequently incubated under normal conditions the resulting infection was even higher than in tubers incubated normally from the beginning. At 39° F. *F. caeruleum* does not make much active growth (Moore, 1945; Lansade, 1949), but it can progress to some extent in April after previous high-temperature storage of the tubers (Table 3). After incubation at 39° F. for 6 weeks no periderm and only a slight suberization was observed on wounded tubers. Weiss, Lauritzen & Brierley (1928) found that below 50° F., even with favourable humidity, tubers cannot form a protective layer which will exclude the more virulent rot organisms, but Starr (1935) concluded that at 4° C. (humidity unstated) a sufficient amount of suberized parenchyma to exclude the dry-rot organisms was produced after 14-20 days although no wound periderm was formed at all at this temperature.

These facts suggest that slow penetration of the fungus at low temperature may occur. The further fact that the resultant infection varies with expected varietal susceptibility also indicates that all these variations are determined primarily by the constitution of the tuber.

Differences in susceptibility can be explained by the fact that the composition of the tuber changes very rapidly with variations in temperature. Higher temperatures have the final effect of hastening the development of sprouting and also increasing the water loss from the tubers, which, Lansade (1949) suggests, is correlated with increase in susceptibility. This applies probably only within a variety. Judging from appearance during storage, Home Guard loses water much more rapidly and to a greater extent than Catriona but the latter variety is much more susceptible to dry rot.

It is probable that such temperature variations have an effect upon susceptibility to dry rot in commercial crops. Variations do occur from year to year, and it has been observed in Britain and in Holland (Mooi, 1950) that a dry season is frequently followed by severe dry-rot outbreaks. One reason which has been suggested for this is that the adherent soil under wet lifting conditions acts as a buffer to protect the potatoes from damage but even so it has been shown (Boyd, 1947) that

lifting damage does not normally play an important part in commercial outbreaks of the disease. A lower level of infection was observed in experimental inoculations in 1950 after a wet growing season, than in the previous dry year (Boyd, 1952*b*). Tubers grown in a year with low rainfall have a lower moisture content than those grown in wet years, and this, according to Lansade (1949), may also play its part. High-soil temperature and low rainfall normally occur together and may combine to produce the same effect of increased susceptibility.

Warm autumn weather would also tend to increase the amount of dry rot in commercial stocks if riddling (the main means of entry of the fungus) is then carried out with the early susceptible varieties. Lower temperatures in potato clamps should thus result in less dry rot than higher temperatures in potato stores, provided subsequent conditions are the same, but lower temperatures during storage after dressing might be expected to be the cause of unexpected development of dry rot fairly late in the storage season, e.g. several months after riddling.

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SOME EFFECTS OF HIGH TEMPERATURE ON THE SUSCEPTIBILITY OF PLANTS TO INFECTION WITH VIRUSES

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(With Plate 15)

When plants were kept at 36° C. for some time before inoculation, their susceptibility to infection by five mechanically transmissible viruses was greatly increased. When kept at 36° after inoculation, fewer local lesions were produced than at lower temperatures, but the effects of the post-inoculation treatment differed with different viruses. Tomato spotted wilt and tobacco mosaic viruses multiply in plants at 36°, and the post-inoculation treatment reduced the local lesions they caused to numbers that varied between 10 and 90% of the control; these two viruses also have large thermal coefficients of heat inactivation. By contrast, tobacco necrosis, tomato bushy stunt and cucumber mosaic viruses, were much affected by post-inoculation treatment, lesion formation being completely prevented by exposure to 36° for a day or more. These three viruses appear not to multiply in plants at 36°, and although they have high thermal inactivation points, they have small temperature coefficients of thermal inactivation.

The extent to which lesion formation was affected by pre- or post-inoculation exposure of plants to 36° depended not only on the length of the treatment, but also on the physiological condition of the plants.

The symptoms of infected plants changed considerably if kept at 36°. At 36° *Nicotiana glutinosa*, inoculated with tobacco mosaic virus, gave chlorotic local lesions instead of necrotic ones, and became systemically infected. When systemically infected plants were brought to ordinary glasshouse temperature, the infected tissues all collapsed and died in a day.

The literature on plant virus diseases contains many references to effects produced by changes in the air temperature at which infected plants are kept. Most references are to effects on the symptoms shown by systemically infected plants, and the commonest is to 'heat masking', the reduction in severity or disappearance of symptoms as the temperature is raised. There is abundant evidence, however, that the optimal temperature for disease expression varies widely both with different viruses and different host plants, so that no generalization is possible. Temperature can also determine whether or not a virus becomes systemic in a host. Samuel (1931) found that, when *Nicotiana glutinosa* were maintained at 35° C., tobacco mosaic virus formed chlorotic lesions and a systemic mottle, instead of the localized necrotic lesions formed at lower temperatures. There has been little work on the effects of temperature in influencing the susceptibility of plants to infection or in influencing the extent to which viruses multiply in infected tissues, although there are suggestions that these may be profound. For example, Best (1936) found, with tobacco

mosaic virus in *N. glutinosa* and tomato spotted wilt virus in *N. tabacum*, that raising the ambient temperature from 15 to 20° increased the numbers of local lesions produced by 30 %; Pound & Walker (1945) found, with both cauliflower mosaic and cabbage black ringspot viruses, that the virus content of extracts from infected plants was highest at temperatures at which symptoms were most severe, and it is known that temperatures around 35° can free systemically infected tissues of some plants from some viruses (Kunkel, 1936, 1941; Kassanis, 1950).

The present paper describes experiments with plants kept at 36°; the results amply demonstrate the varied effects high temperature can have with different viruses and hosts, but they also suggest that exposing plants to high temperatures before inoculation may be a method of greatly increasing susceptibility to many viruses.

MATERIALS AND METHODS

So that results could be expressed quantitatively, the viruses and hosts used were mainly those that produce countable local lesions. They were: tobacco mosaic virus in *N. glutinosa* L. and *Phaseolus vulgaris* L. var. Riscardo de Milho; the Rothamsted culture of tobacco necrosis virus in *P. vulgaris*, var. Prince and *Nicotiana tabacum* L. var. White Burley; tomato bushy stunt virus in *N. glutinosa*; a severe strain of cucumber mosaic virus in *Phaseolus vulgaris*, var. Prince; tomato spotted wilt virus in *Nicotiana tabacum*.

The heated plants were in a glass incubator kept in the glasshouse. The incubator was a metal frame 45 cm. high, 60 cm. wide and 90 cm. long, had a metal floor and glass sides and top. It was not air-tight as the glass panels did not fit tightly to the frame. It opened from the top, and the four 230 W. electric heaters were fixed two on each of the longest sides, near the sides and 12 cm. from the floor. The temperature was controlled by a thermostat, the sensitive element of which was inserted through a side wall and reached the centre of the incubator. A thermometer was placed close to it.

Ten small or eight large (5 and 7 in. in diameter) pots with plants could be treated at a time; they stood in a trough of water, which occupied the entire floor of the incubator. The thermostat was adjusted at 36° and the temperature in different parts of the incubator usually did not vary beyond the range of 35–37°. When the sun was shining, the blind of the glasshouse was lowered, but in summer the temperature of the incubator sometimes temporarily rose to 38°. The control plants were kept in the same glasshouse in which no attempt was made to control the conditions. The temperatures fluctuated but were usually between 18 and 23°.

It seems reasonable to assume that the effects obtained by keeping plants in the heated incubator were mainly, and probably entirely, the results of the high temperature. Light intensity and relative humidity did not differ greatly from conditions in the glasshouse, and plants left in the incubator when the heaters were not working reacted similarly to plants kept on the benches in the glasshouse.

To reduce errors from individual plant variation, care was taken to select plants not only of the same age but also of the same appearance. *N. glutinosa* plants were usually trimmed to six leaves, tobacco plants to three, and the bean plants were used at the two-leaf stage.

EFFECT OF TEMPERATURE ON THE NUMBERS OF LOCAL LESIONS

Pre-inoculation treatment

The numbers of local lesions, with all the viruses and plants which were tested, increased when the plants were kept at 36° before inoculation (Table 1). As little as 6 hr. heating caused an increase in susceptibility to infection, and the maximum was usually reached after 2 days. The time taken for maximal response depended a great deal on the age and general physiological state of the plants. The older the plants were, the longer they had to be treated. The most constant results were obtained with beans, which were always used at the same stage of growth.

TABLE 1. *The increased susceptibility to infection caused by pre-treatment of plants at 36°*

Virus-plant combination and concentration of inoculum	Length of treatment					
	Untreated	6 hours	1 day	2 days	3 days	4 days
Tobacco necrosis virus in beans var. Prince, 1 mg./l.	2*	4	29	46	—	—
Tobacco mosaic virus in <i>Nicotiana glutinosa</i> , 5 mg./l.	32	—	98	111	141	171
Tomato bushy stunt virus in <i>N. glutinosa</i> , 50 mg./l.	18	84	83	97	—	—
Tomato spotted wilt virus in tobacco, undiluted tobacco sap with Celite	86	157	197	165	—	—

* Average number of lesions per leaf.

The age of the plant also affected the extent to which the numbers of lesions were increased. To quote one experiment in which two lots of beans differing in time of sowing by 10 days were heat-treated for 0, 1 and 2 days before inoculation with tobacco necrosis virus 1 mg./l., the average numbers of lesions per leaf for the three periods of exposure to heat were respectively 22, 48, 27 for the younger plants and 3, 28 and 40 for the older.

Post-inoculation treatment

There was no qualitative difference between different viruses when the host plants were kept at 36° before inoculation; all viruses in all hosts produced more lesions than on untreated plants. There were, however, striking differences when plants were kept at 36° after inoculation. With the five viruses that were tested, the numbers of local lesions were reduced, but whereas with tobacco mosaic and tomato

spotted wilt viruses the reduction was small, with tobacco necrosis, tomato bushy stunt and cucumber mosaic viruses lesion formation could be entirely inhibited by keeping the plants at the high temperature. The extent to which lesions were reduced varied with individual viruses and with the length of time the plants were kept at 36°.

The numbers of local lesions produced by tobacco necrosis and cucumber mosaic viruses in beans were greatly reduced by a few hours, and a day at 36° wholly prevented infection leading to lesion production (Table 2). Similar results were obtained with tobacco necrosis virus in treated tobacco plants, showing that the effect was independent of the test plant.

TABLE 2. *Reduction of local lesions when bean plants are kept at 36° after inoculation*

Viruses and concentration of the inoculum	Length of treatment (hr.)					
	0	1	2	4	6	24
Tobacco necrosis, 5 mg./l.	69*	38	16	—	4	0
Cucumber mosaic, undiluted sap from infected tobaccos	97	132	116	49	4	0

* Average number of lesions per leaf.

Tobacco bushy stunt virus in *N. glutinosa* behaved similarly to tobacco necrosis virus except that longer exposures at 36° were needed to inhibit lesion production completely. Plants inoculated with tomato bushy stunt virus at 50 mg./l. formed an average number of sixty lesions per leaf in the untreated controls, and 25, 9, 2 and 1 in plants heated for 1, 2, 3 and 4 days respectively. The lesions which appeared in plants left for 2 or more days at high temperature appeared later and developed more slowly than in the control plants.

It is known that tobacco necrosis, tomato bushy stunt and cucumber mosaic viruses have a small thermal coefficient of heat inactivation and that they lose most infectivity when heated *in vitro* at temperatures well below their thermal end-points. However, the reduction in the numbers of local lesions in the heated plants is unlikely to be a direct result of this property, for periods of heating plants that are adequate to reduce lesions had no effect on tobacco necrosis virus *in vitro*. This was shown by diluting a virus preparation in different fluids and keeping them at 36°. Samples, taken at different times and stored in the refrigerator, were tested for their infectivity on bean leaves (Table 3). The infectivity of the virus in water was reduced but not to the same extent as were lesion numbers when the inoculated plants were kept at 36°. Crude sap and phosphate buffer pH 7 protected the virus from the inactivation.

Exposing *N. glutinosa* to 36° after inoculation with tobacco mosaic virus gave very variable results. Sometimes heated plants produced as many lesions as the control, but more often the numbers were reduced by different proportions up to 50 %. The variability in the results between experiments was found to be caused by differences in age of the plants and general state of susceptibility.

Table 4 gives the results of a test using two concentrations of tobacco mosaic virus, to compare *N. glutinosa* plants of two ages, with beans var. Riscardo de Milho. This plant is much more resistant to infection than *N. glutinosa* and to obtain comparable numbers of lesions per leaf tobacco mosaic virus needs to be a thousand times more concentrated. Heating had the greatest effect in increasing the susceptibility of the beans, an intermediate one with the older *N. glutinosa* plants and least in young plants, i.e. the effect was most pronounced with the most resistant host plants. Also it was greater with the higher concentration of the inoculum (Table 4). Assuming that the plants have regions of different susceptibility, which require varying amounts of virus to become infected (Kleczkowski, 1950), heat seems to affect those regions which require more virus for an infection to begin.

TABLE 3. *Effect of heating tobacco necrosis virus 5 mg./l. at 36° in different conditions*

Media	Length of treatment (hr.)			
	0	1	3	6
Water	79*	58	36	35
Crude sap from bean diluted to one-half	49	60	59	52
Phosphate buffer M/30, pH 7	55	59	44	51

* Average number of lesions per bean leaf.

TABLE 4. *Effect of one day at 36° after inoculation of beans and Nicotiana glutinosa plants of two ages*

Type of plant	Average number of lesions from 20 leaves		
	Concentration of inoculum (mg./l.)	Plants at 36° for 1 day	Untreated plants
Young <i>N. glutinosa</i>	5	19	25
Young <i>N. glutinosa</i>	1	5	6
Older <i>N. glutinosa</i>	5	8	18
Older <i>N. glutinosa</i>	1	3	2
Bean var. Riscardo de Milho	1000	3	28
Bean var. Riscardo de Milho	100	2	13

While testing the effect of post-inoculation heat treatment on tobacco mosaic virus in *N. glutinosa*, it was found that treatments longer than 1 day did not further decrease the numbers of lesions. With tomato spotted wilt virus, however, there was a continuing decrease up to 4 days' heating, and the percentage of reduction was much larger than with tobacco mosaic virus. For example, in a typical experiment, tobacco plants inoculated with tomato spotted wilt virus, with the aid of celite, were heat treated for 0, 1, 2 and 4 days and the mean numbers of local lesions per leaf, were 542, 191, 92 and 57 respectively. The lesions were already visible in 4 days at high temperature and longer exposures had no further effect on their numbers.

Tests were made with three viruses to find the maximum delay between inoculation and exposure to 36° that would permit an effect on lesion numbers. Table 5 shows that as long as the lesions in the control plants were not formed heat treatment could be postponed and still reduce their numbers. The treatment was stopped when lesions appeared in the unheated plants, and the plants were kept under observation for a few days to ensure that any delayed lesions would be recorded and counted. When beans infected with tobacco necrosis or cucumber mosaic viruses were kept at 36° when lesions were beginning to appear, the lesions remained small while the plants were at 36°, but increased in size when the plants were later kept at the lower temperature. It seems likely that the virus ceased multiplying during the treatment, but the period of exposure was not long enough to inactivate the virus already formed in the lesions. By contrast, lesions produced by tomato spotted wilt virus increased in size more rapidly at 36° than at ordinary temperature.

TABLE 5. *Reduction of local lesions with bean and tobacco plants kept at different times after inoculation at 36°*

Viruses and concentration of inoculum	Time in days from inoculation when treatment began					Untreated plants	Time in days after inoculation when the lesions appeared in the untreated plants
	0	1	2	3	4		
Tobacco necrosis virus, 7.5 mg./l.	0*	5	8	26	—	27*	2
Cucumber mosaic virus, undiluted sap from tobacco	0	0	29	123	—	144	3
Tomato spotted wilt virus, undiluted sap from tobacco with celite	192	243	131	—	332	346	4

* Average number of lesions per leaf.

SYMPTOMS AND VIRUS CONCENTRATION IN HEATED PLANTS

Samuel (1931) was the first to observe that at high temperature tobacco mosaic virus produced in *N. glutinosa* chlorotic instead of necrotic local lesions, and then became systemic. In my experiments with tobacco mosaic virus, the local symptoms have varied; sometimes there were no lesions formed but only a general yellowing of the inoculated leaves, at other times there were separate lesions, either chlorotic local lesions, with or without a fine necrotic ring, or concentric chlorotic rings and other patterns of the ringspot type (Pl. 15, figs. 1-4). The causes of these variations were not investigated. Symptoms of systemic infections were always obvious in young *N. glutinosa* plants 2 days after inoculation. Systemically infected leaves contained crystalline inclusions, similar to those found in infected tobacco plants kept at ordinary temperature.

Although there was no necrosis when infected *N. glutinosa* plants were kept at 36°, when such plants were exposed to ordinary glasshouse conditions, the infected tissues became necrotic and collapsed in about a day (Pl. 15, fig. 5). The size of the

collapsed areas increased with longer heat treatment and plants which were systemically infected with the virus, when brought to ordinary glasshouse temperature, wilted and died (Pl. 15, figs. 6, 7).

When *N. glutinosa* plants with fully developed necrotic local lesions were later exposed to high temperature, the virus moved from the necrotic zone and became systemic. At ordinary temperature localization of tobacco mosaic virus in these plants is generally thought to be a consequence of necrosis, the virus being considered as unable to move from dead to living cells. It seems, however, that additional factors must also be involved, for at 36° the virus moves readily from areas of dead cells into living ones. That other factors are involved is also suggested by the fact that many viruses are not localized in hosts where they cause local necrosis and the several examples in the literature of viruses multiplying locally without becoming systemic and without causing necrosis (Holmes, 1941; Bawden & Kassanis, 1947; McKinney & Fulton, 1949).

Best (1936) has shown that the necrotic lesions produced by tobacco mosaic virus in *N. glutinosa* are surrounded by haloes that fluoresce in ultra-violet light, and that this results from the accumulation there of scopoletin, a substance that occurs in small quantities in uninfected tissues. Observations on infected plants at different temperatures show that the accumulation of scopoletin is closely associated with necrosis. Observations were made both by examining intact leaves in ultra-violet light and extracts from the leaves that had been clarified by heating to 60° for 10 min. and centrifuged. Fluorescence was detected only in plants that had been kept at ordinary glasshouse temperatures long enough for necrotic symptoms to develop, and samples taken direct from plants at 36°, which were showing chlorotic symptoms, did not fluoresce. There was no correlation between fluorescence and virus multiplication. Thus sap from plants kept continuously in ordinary glasshouse conditions fluoresced strongly but contained too little virus to give a detectable precipitate with virus antiserum, whereas sap from chlorotic plants kept in the incubator did not fluoresce but precipitated when diluted as much as 1/128. When such heated plants were placed at a lower temperature, the fluorescence rapidly increased as the lesions became necrotic, without any corresponding increase in virus. There is no evidence to show whether the accumulation of scopoletin causes the necrosis or whether it accumulates because the cells die.

The change from localized to systemic infection caused when *N. glutinosa* plants infected with tobacco mosaic virus are kept at high temperature, is not a general phenomenon applicable to other hosts or other infections. In the bean variety Riscardo do Milho, for example, tobacco mosaic virus continued to produce only necrotic local lesions, although these were larger than in control plants. The various types of local lesions produced by different strains of tobacco mosaic virus in cucumber are the same at 36° as at lower temperatures (McKinney & Fulton, 1949), and the virus does not become systemic in plants kept at the high temperature. Systemic invasion of tobacco plants with tomato spotted wilt virus was apparently

prevented by keeping the plants at high temperatures. The type of local lesion was altered; instead of circular areas of leaf collapsing, the lesions consisted of a series of concentric rings, mainly chlorotic with a superficial etching. The lesions appeared earlier than in control plants, enlarged more rapidly and reached greater dimensions, sometimes becoming 1 in. in diameter. Like the lesions on control plants, these fluoresced in ultra-violet light, and extracts from the leaves were more infective than extracts from control leaves with similar numbers of local lesions.

It was shown above that exposure to 36° after inoculation prevented tomato bushy stunt virus from forming necrotic local lesions in *N. glutinosa*. To see what effect exposure would have on systemically infected plants experiments were made with *Datura stramonium* and tomato (*Lycopersicum esculentum* Mill. var. Kondine Red).

Datura stramonium plants, showing severe systemic symptoms, were placed in the incubator for periods of 0, 2, 4, 8 and 16 days. Two plants were used for each treatment. The plants which were heated for 8 and 16 days produced more leaves than the others, and these extra leaves were apparently healthy. At the end of the experiment all the leaves were picked and the extracted sap was heated for 10 min. at 60° and centrifuged. The virus concentration in the sap was then assayed serologically. The sap from plants that received the different treatments gave precipitin titres of 1/64, 1/32, 1/16, 1/8, 1/8 respectively. This showed that the concentration of the virus in sap was less from plants kept at 36°, but no definite conclusions can be drawn from this experiment as the new leaves produced by the heated plants may have been free from virus and diluted the total amount present originally in the plant. Infectivity tests made with *Nicotiana glutinosa* from the different saps, undiluted and diluted 1/10, were unsatisfactory. The saps from plants exposed for 8 and 16 days to 36° produced no lesions, but sap from the control plants also produced only a few; this is probably because the sap from *Datura stramonium* contains a strong inhibitor of the infectivity of plant viruses (Kassanis & Kleczkowski, 1948).

Similar tests were made with tomatoes. Plants systemically infected with tobacco bushy stunt virus and showing severe symptoms were heated for 0, 4, 8 and 16 days. The plants with the longest treatment again produced most new leaves which were symptomless. Plants were harvested after 16 days, and leaves which grew during the experiment were separated from those present at the beginning. Sap was extracted from the different samples of leaves and inoculated at various dilutions to *Nicotiana glutinosa* for lesion counts; samples were also clarified by heating to 60° and centrifuging, and then tested for their precipitation end-points with virus antiserum (Table 6).

The precipitin titres and the infectivity were both less in the sap from heated plants than from the controls and the differences increased with increases in the period of heating. The old and new leaves behaved similarly, suggesting that the heating led to the disappearance of virus from tissues in which it was already

established. The fall in infectivity is proportionally greater than the fall in serological activity, showing that the ratio of infective to non-infective particles is smaller in sap from the heated than in sap from control plants. Heating *in vitro* at temperatures below those at which preparations of tomato bushy stunt virus lose their serological activity, also destroys infectivity (Bawden, 1950).

TABLE 6. *The virus concentration of sap from tomato plants systemically infected with tomato bushy stunt virus after different periods at 36°*

Length of treatment in days	Serological titre		Total number of local lesions on 6 leaves of <i>N. glutinosa</i>							
			Young leaves				Old leaves			
	Young leaves	Old leaves								
			Dil. 1/1	1/10	1/100	1/1000	1/1	1/10	1/100	1/1000
0	1/64	1/8	—	—	101	24	—	—	23	14
4	1/32	1/8	—	69	22	5	—	11	5	2
8	1/2	1/8	23	3	4	—	23	5	8	—
16	1/2	1/1	0	1	0	—	1	3	0	—

The apparent disappearance of virus from systemically infected leaves when plants are kept at 36°, has three equally plausible explanations. First, the virus may be destroyed at the high temperature; its stability *in vitro* is such that it is unlikely this would happen as a direct result of the temperature, but host-cell mechanisms that destroy it may be brought into operation at 36°. Secondly, virus may normally be in a state of equilibrium with host proteins, so that its concentration is kept approximately constant only by continuous production of new virus, and production at 36° may be so reduced as to fall below the level needed to replace normal losses. Thirdly, heating may not reduce the total quantity of material with the serological characters of the virus, but may alter its distribution, so that less is extracted in the sap from heated plants.

Periods of heating for longer than 16 days were not tested in these experiments, but the results in Table 6 suggest that prolonged treatment at 36° might have freed tomato and *Datura stramonium* plants from tomato bushy stunt virus.

DISCUSSION

The effects on susceptibility to viruses of keeping plants at 36° resemble in some ways those of keeping plants under reduced light intensity. Bawden & Roberts (1947, 1948) found that shading plants for some days, or keeping them in darkness for shorter periods, immediately before inoculation increased susceptibility to infection. Keeping plants in darkness after inoculation had relatively little effect, but usually reduced the numbers of lesions produced. One obvious effect of keeping plants in darkness is to reduce the carbohydrate content of leaves and the carbon/nitrogen ratio. Bawden & Roberts found no evidence that the carbohydrates acted as inhibitors of infection when added to viruses *in vitro*, but they

suggested as one possible explanation of the increased susceptibility that the carbohydrates were interfering with the establishment of the virus particles in the cells.

Similarly, keeping plants at 36° reduces the carbohydrates. For example, one estimation made in November with tobacco plants showed a reduction of 40 % in total carbohydrates after 24 hr. at 36°, but the reduction may have been less at other times of the year. Plants in winter become chlorotic after few days' treatment, whereas in summer they showed no injury and made good growth when kept for several weeks at 36°. However, many other changes also occur when plants are placed in the dark or at high temperature, and the increased susceptibility is just as likely to be the result of one of these changes as to changes in carbohydrates. For example, considerable alteration of the nitrogenous components of the cells have been reported in tobacco leaves kept in the dark (Vickery, Pucher, Wakeman & Leavenworth, 1937).

With tobacco mosaic and tomato spotted wilt viruses the effect of high temperature as post-inoculation treatment was greater but comparable with that of putting the plants in the dark. With tobacco necrosis, tomato bushy stunt and cucumber mosaic viruses, however, it was striking, for infection with these could be completely inhibited.

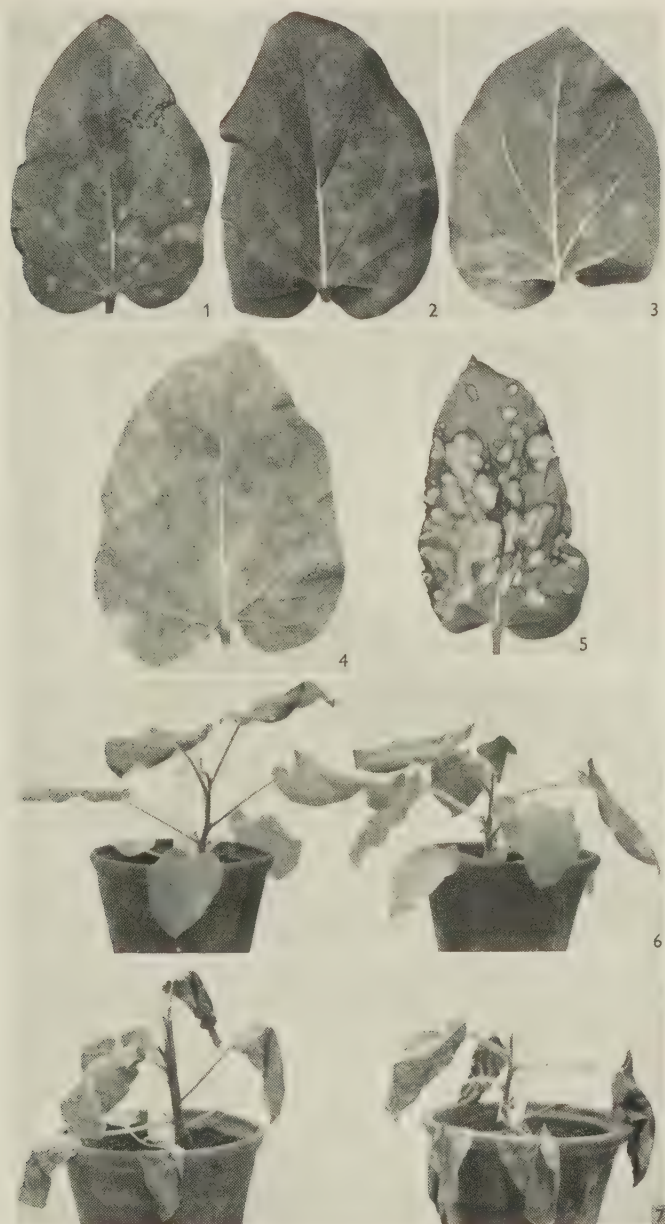
The five viruses tested seem, therefore, to divide into two groups according to their behaviour in plants at 36°, one group multiplying and the other apparently not. There is no correlation between their behaviour in plants at 36° and their thermal inactivation points, i.e. the minimum temperature at which heating for 10 min. inactivates the virus *in vitro*. Tomato bushy stunt virus, for example, has a thermal inactivation point of about 80°, and it formed no lesions on plants kept at 36°, whereas tomato spotted wilt virus with a thermal inactivation point of 45° (Best, 1946) formed lesions at 36°. On the other hand, there is a correlation between the viruses of the two groups and their temperature coefficient of thermal inactivation *in vitro*. Tomato bushy stunt (Bawden, 1950), tobacco necrosis (Bawden, 1941) and cucumber mosaic viruses (Bhargava, 1951) have small temperature coefficients and lose much infectivity when heated at temperatures well below their thermal inactivation points, whereas tobacco mosaic (Price, 1933) and tomato spotted wilt viruses (Best, 1946) have large temperature coefficients and loss of infectivity occurs in a small range near the thermal end-point. Too few viruses have been studied, however, to say if the correlation between inactivation *in vitro* and behaviour in the plants at 36° is a general one, or only a coincidence.

Heating has been the only practical therapeutic treatment in freeing plants from virus infection. It has succeeded with several viruses, but as none of these has been transmitted mechanically, nothing is known about their behaviour when heated *in vitro*. Bawden (1950) has suggested that these need not necessarily be viruses with low thermal inactivation points, and the results described in this paper agree with the view that they may resemble tomato bushy stunt virus in having small thermal coefficients of heat inactivation.

It is a pleasure to thank Dr R. H. Bradley, now at the Division of Entomology (Fredericton, New Brunswick), who gave me much assistance at the start of the work.

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KASSANIS—*High temperature and susceptibility of plants to virus infection*

EXPLANATION OF PLATE 15

Figs. 1-5. *Nicotiana glutinosa* leaves, inoculated with tobacco mosaic virus, from plants treated differently and showing:

Fig. 1. Necrotic local lesions produced at glasshouse temperature, 5 days after inoculation.

Fig. 2. Chlorotic local lesions, some with necrotic rings at 36° C., 5 days after inoculation.

Figs. 3, 4. Chlorotic local lesions of the ringspot type, one week after inoculation at 36°. Leaf of fig. 4 inoculated with more concentrated inoculum.

Fig. 5. Chlorotic local lesions which collapsed and became necrotic. The plants remained 3 days at 36° and 2 days at glasshouse temperature after inoculation.

Fig. 6. Two *N. glutinosa* plants inoculated with tobacco mosaic virus and kept for 8 days at 36°.

Fig. 7. The same plants as Fig. 6 after being kept for 2 more days at glasshouse temperature. All tissues where virus had multiplied collapsed and died at lower temperature.

(Photographs by Mr V. Stansfield, F.R.P.S.)

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STUDIES OF FLUCTUATIONS IN INSECT POPULATIONS

XII. FURTHER EVIDENCE OF PROLONGED LARVAL LIFE IN
THE WHEAT-BLOSSOM MIDGES

BY H. F. BARNES

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(With 1 Text-figure)

Contarinia tritici emerges the same year as the larvae are in the wheat ears and up to 3 years later. Its parasites emerge up to 2 years after the larvae are in the wheat ears, but never the same year. This indicates that the supernumerary generation of *C. tritici* that occurs on couch grass in August and September may be expected to be free from parasites and so constitutes a dangerous source of replenishment of the pest.

Emergence of *Sitodiplosis mosellana* can take place after the larvae have been in the soil up to twelve winters. Most of any one year's larvae do not necessarily emerge as midges after one winter, although usually this is so. Any one year's flight is probably a composite one derived from several previous generations of this one-generation-a-year gall midge.

INTRODUCTION

In the tenth of these studies (Barnes, 1943) it was shown that under insectary conditions *Contarinia tritici* Kirby emerged in the autumn of the same year as the larvae had attacked the wheat, or during the next spring or after a lapse of two winters. Its parasites emerged after one and two winters. The other wheat blossom midge, *Sitodiplosis mosellana* Géhin, never emerged the same year as the larvae fed in the wheat ears but only in the spring up to 4 years after the larvae had left the plant. Its parasites also emerged after one or two winters, and this variability in the duration of the larval stage could assist in the survival of the species and be a factor in the appearance of sudden increases in population. Further evidence of this prolonged larval life in these two gall midges is now presented.

Each year the larvae of both species have been placed in fibre soon after extraction from the wheat ears and then kept in lamp glasses standing in pots of soil and covered by muslin sewn on to an iron ring. These rearing cages have been kept in an outdoor unheated insectary. Samples of larvae have been taken each year from the Rothamsted field of permanent wheat, Broadbalk, and retained in the insectary until no more emergence of the midges could be reasonably expected.

Contarinia tritici

Table 1 gives the numbers of *C. tritici* and its parasites that have emerged from larvae collected each summer including and since 1939. It will be seen first that in 10 out of the 13 years there has been a slight emergence of midges the same year

TABLE I. Emergence of *Contarinia tritici* and its parasites

[illegible]

TABLE 2. *Emergence of Sitodiplosis mosellana and its parasites*

[illegible]

as the larvae were feeding in the wheat ears; in fact, only in 4 out of the last 27 has there been no such emergence. Secondly, that most midges and parasites have always emerged the subsequent year. Thirdly, that whereas in one case (1939 sample) midges finished emerging after one winter in the fibre, in six cases (1940, 1941, 1942, 1944, 1945 and 1946 samples) midges continued to appear after the second winter, and in three instances (1943, 1947 and 1948 samples) midges emerged even after three winters. In no instance so far has any emergence taken place more than three winters after the larvae were on the wheat.

Parasites have never emerged the same year as the larvae have been extracted from the wheat ears but have appeared after one (five times) and two (six times) winters. In no case have parasites emerged after more than two winters in the fibre.

Thus if insectary emergence is the same as that which occurs in the field, the flight of *C. tritici* which occurs in any one year may consist of individuals that were larvae either 1, 2 or 3 years previously, while that of the parasites may be derived from either the previous year or the one preceding it.

Sitodiplosis mosellana

Table 2 shows the numbers of *S. mosellana* and its parasites that have emerged from samples of larvae collected annually since 1939. It will be seen that this species differs markedly from *Contarinia tritici* in that there has never been any emergence the same year as the larvae were collected and that emergence continues over a much greater number of years. In fact, up to 1951 one individual has emerged 12 years after the larva was taken from the food plant, another has emerged after 11 years in the fibre, two specimens have appeared after 10 years and fifteen individuals after 9 years. It is not known how long midges will go on emerging, but it seems probable that the maximum life of the larvae is not much more than 12 years. It might have been expected that the emergence from any one year's sample of larvae would have declined steadily from year to year, but this is not so. For instance, more midges emerged from the 1939 larvae in 1943 than in either 1941 or 1942.

TABLE 3. *Frequency (1939-48) of maximum and next-to-maximum emergence in relation to numbers of winters passed after the larvae have left the wheat ears*

Numbers of winters spent in fibre	Frequency of maximum emergence	Frequency of next-to- maximum emergence
1	6	1
2	2	4
3	2	2
4	0	2
5	0	1

Other similar occurrences are shown in Table 2. Table 3 shows the frequency of the maximum and next-to-maximum emergence. Taking the total emergences over the first 10 years of the observations it is seen that most midges emerged after one winter in the fibre, fewer after two winters, fewer still after three and so on, but

this is not always so, e.g. in 1947 only 53 midges from the 1946 sample emerged whereas the following year, 1948, 335 emerged.

Parasites of *Sitodiplosis mosellana* have continued emerging up to 6 years after the larvae were collected, e.g. in 1947 from the 1941 sample and in 1951 from the 1945 sample.

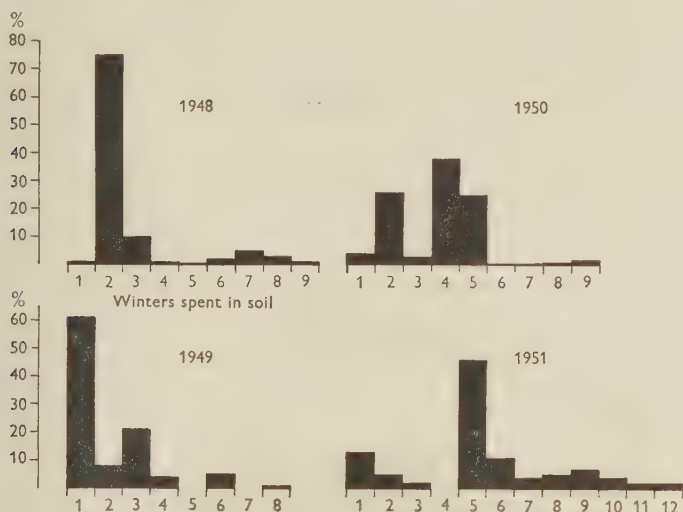


Fig. 1. Percentage composition of the 1948, 1949, 1950 and 1951 emergences of *S. mosellana* in the insectary.

The result of this prolonged larval life of the midge and its parasites is that any one year's flight may be composed of varying numbers of midges derived from the previous 12 years' larvae (Fig. 1) and of parasites arising from the previous 6 years. The chances of brother-sister mating is thus lessened. It might also be an explanation of heavy infestations of wheat on land that has not been under wheat for several years, although of course the midges are under certain conditions carried by the wind some distance. The actual numbers of midges reared in the insectary are small, but the extent to which this prolonged life of the wheat blossom midges takes place in the field has now been adequately demonstrated by Golightly (1952).

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STUDIES OF FLUCTUATIONS IN INSECT POPULATIONS

XIII. AN IMPROVED METHOD OF ASCERTAINING THE CORRECT DATE TO SAMPLE WHEN ASSESSING LARVAL INFESTATIONS OF THE WHEAT-BLOSSOM MIDGES

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(With 2 Text-figures)

Fully fed larvae of the wheat-blossom midges need moist conditions in order to descend from the ears to the soil. A dry spell coinciding with the fully fed stage therefore causes an accumulation of larvae, which subsequently leave the ears in a body when rain comes. Conversely, in wet weather each larva leaves the wheat as soon as it finishes feeding, and, since all the eggs are not laid on the same day, the descent will then be a gradual process.

A method is described of taking small samples of wheat ears at frequent intervals, thus facilitating the determination of the correct date on which to take the main sample for the assessment of infestation by the two wheat-blossom midges.

INTRODUCTION

The study of fluctuations in the numbers of larvae of *Contarinia tritici* Kirby and *Sitodiplosis mosellana* Géhin attacking the ears on the Rothamsted field of permanent wheat has now been continued for 25 successive years. Considering both species together there have been peaks of abundance every 5 years, namely in 1931, 1936, 1941, 1946 and 1951. On Broadbalk in 1936 and 1951 there were 'depressed peaks', purely local occurrences preventing actual peaks.

From 1927 to 1940 the sample of 500 ears of wheat (fifty from each of ten plots), on which the assessment of the yearly numbers on Broadbalk was based, was collected about 3 weeks after the crest of emergence of the midges (Barnes, 1932). Three weeks is the average interval between oviposition and the time when the larvae have completed their growth in the ears. The approximate date on which to sample was ascertained by observing daily emergences in the insectary and comparing them with the numbers of midges to be seen ovipositing in the field. The exact date was finally chosen each year by examining several ears to find what stage of growth the larvae had reached. The object was to sample when the maximum number of larvae were practically full grown and ready to leave the ears for the soil where they spend the period between completing their feeding and pupation. Owing to the fact that larvae can leave the ears only when they are wet, this method of ascertaining the correct date proved too uncertain, since the decision to sample the next day might be taken, and then overnight a shower of rain might

come with the result that the majority of the larvae would suddenly leave the wheat ears; giving too low a figure of infestation.

An improved method of ascertaining the correct date on which to sample for larval infestations is now described, and observations are given on the speed of larval descent.

THE IMPROVED METHOD

In 1941 a system of 'pre-sampling' was initiated which has been continued each subsequent year. This necessitates taking samples of fifty ears only from one plot at frequent intervals. The result is that several figures for the high numbers of larvae in the ears are obtained and the time of descent of the larvae from the ears is revealed. If it has been feasible to take the full sample of 500 ears when the peak number of larvae is still in the ears this of course has been done. On the other hand, if rain or any other reason has prevented the main sample from being taken until after the descent has started, the figures obtained in the 'pre-sampling' of the one plot enable the low figure obtained for the ten plots to be corrected. From 1941 to 1945 inclusive plot 2 was pre-sampled, from 1946 to 1950 plot 14 and in 1951 a 5-year period of pre-sampling plot 11 was initiated. Different plots have been chosen for the 5-year periods since the time of ear emergence may vary slightly from plot to plot.

The same information is collected in the 'pre-sampling' as in the main sample (Barnes, 1932, 1941), but only the numbers of grains attacked, the total numbers of larvae of both species and the average number of *Contarinia tritici* per infested grain are of direct use in establishing the correct date for the main sample. The number of blind spikelets serves to indicate the general evenness of the samples and shows that fifty ears are enough for this purpose. The descent of the larvae is indicated by the average number of *C. tritici* per grain (it is likely to be less accurate towards the end of the descent when very few grains still contain larvae), by the numbers of grains infested by both species and by the total numbers of larvae of both species. The latter figures give an early indication of the infestation to be expected in the main sample.

Since oviposition extends over some weeks, if the 'pre-sampling' is started too early when there are still eggs and minute larvae present, some are liable to be overlooked. When all the larvae are large enough to be seen the number of infested grains and total numbers of larvae may be expected to remain relatively constant for a few days until the oldest larvae become full grown and start leaving the ears. Because of earlier egg-laying the descent of *C. tritici* may be expected to start before that of *Sitodiplosis mosellana*.

RESULTS

The full results of the 'pre-sampling' from 1941 to 1951 are available for consultation in the Entomology Department, Rothamsted Experimental Station. Table 1 gives the results of 'pre-sampling' in 1943. In that year, 21 June proved rather

early to start sampling, but 7 days later the larvae of both species were large enough to ensure that all were counted. High numbers of *Contarinia tritici* remained in the ears until 3 July and the main sample was safely taken on that date. Three days later there were indications that the descent had started. This was established on 10 July; in spite of the greater number of infested grains the average number of larvae had by this date fallen to nine. By now *Sitodiplosis mosellana* had started leaving the ears as was confirmed on 13 July. By this date practically all the *Contarinia tritici* had left, whereas there was a considerable proportion of the *Sitodiplosis mosellana* still in the ears at least up to 15 July when the last 'pre-sample' was taken.

TABLE 1. *Pre-sampling (50 ears), plot 2, 1943*

Date	No. of grains infested by		Total larvae		Av. no. of <i>C. tritici</i> per infested grain	No. of blind spikelets
	<i>C. tritici</i>	<i>S. mosellana</i>	<i>C. tritici</i>	<i>S. mosellana</i>		
21 June	5	43	55	61	11	157
28 "	7	137	106	207	15	159
30 "	12	122	166	158	14	175
2 July	10	145	123	234	12	171
3 "**	9	128	122	186	14	176
6 "	6	137	64	199	11	194
10 "	15	103	144	139	10	207
13 "	4	71	9	96	2	208
15 "	1	72	1	89	1	205

* Date is that on which the year's main sample of 500 ears was taken.

Table 2. *Pre-sampling (50 ears), plot 2, 1944*

Date	No. of grains infested by		Total larvae		Av. no. of <i>C. tritici</i> per infested grain	No. of blind spikelets
	<i>C. tritici</i>	<i>S. mosellana</i>	<i>C. tritici</i>	<i>S. mosellana</i>		
6 July	12	4	142	5	12	146
8 "	13	5	157	6	12	173
10 "	9	5	84	6	9	163
11 "**	12	3	45	6	4	185
14 "	13	3	59	3	5	158
17 "	9	4	26	4	3	152
19 "	7	0	28	0	4	156
21 "	2	2	8	3	4	168

* Date is that on which the year's main sample of 500 ears was taken.

Table 2 gives the results of 'pre-sampling' in 1944. In that year, 6 July proved to be rather late to start sampling since only two samples were obtained when the numbers of *Contarinia tritici* were at their height. The descent started between 8 and 10 July; consequently, the main sample was not taken that year at the time when the greatest numbers of larvae were present in the ears. The usefulness of studying two species was also apparent in 1944.

DISCUSSION

The initiation and continuation of the larval descent depends to a great extent on the amount of rain that occurs when the larvae have become full grown. If there is a spell of dry weather the larvae appear to be unable to descend, and an accumulation of full-grown larvae in the ears occurs. Then when rain does come there is a sudden descent. Fig. 1 illustrates the descent of *C. tritici* in 1949 and 1950, while Fig. 2

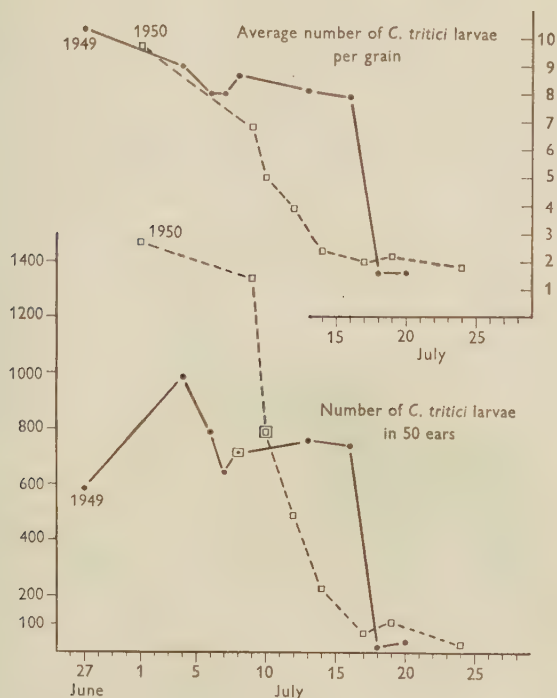


Fig. 1. Larval descent of *C. tritici* in 1949 when there was a dry spell during July, and in 1950 when there was not a dry spell in July. The main sample was taken on 8 and 10 July respectively.

deals with *Sitodiplosis mosellana* in the same years. The figures are those obtained by 'pre-sampling' plot 14, and it is probable that in 1949 on 27 June some of the larvae of both species were too small to be seen. By 4 July sufficient growth had taken place for all to be counted. From then until 16 July, no appreciable rain fell, the numbers of larvae of both species remained constant and the average number of *Contarinia tritici* only varied between 9.0 and 7.9. Rain then came, and when the next sample was taken on 18 July the larvae had descended

and the numbers of *C. tritici* had dropped from about 700 to about 20-40 and those of *Sitodiplosis mosellana* from about 50 to about 20. The average number of *Contarinia tritici* also fell suddenly from about 8 per grain to about 1.5. In 1950, on the other hand, there was not a dry spell in July. In this year, as the larvae of *C. tritici* became full grown they descended to the soil, i.e. from 1 July onwards gradually. Those of *Sitodiplosis mosellana* were too small for all to be seen on 1 July, started leaving the ears about 11 July and subsequently their descent was also gradual.

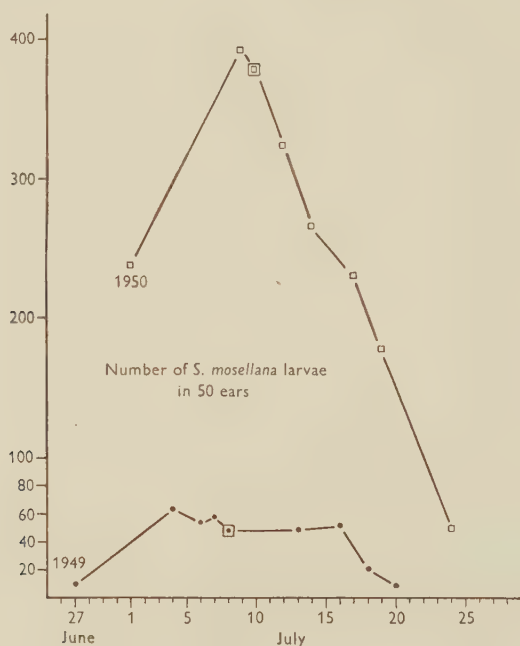


Fig. 2. Larval descent of *S. mosellana* in 1949 and 1950.
See Fig. 1 for further details.

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SOIL SAMPLING FOR WHEAT-BLOSSOM MIDGES

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(With 1 Text-figure)

A method of assessing the population of wheat-blossom midges by the recovery of the cocoons from soil is described.

In the dry summer of 1947, considerable numbers of larvae of *Sitodiplosis mosellana* Géhin were still in the ears at the time of harvesting. Burning the straw immediately after combining may considerably reduce the numbers of midges.

The majority of the larvae are found in the top 3 in. of the soil although a considerable number may be found from 3 to 6 in. Headland infestations may be lighter than the rest of the field.

Cocoons of *S. mosellana* remain viable in the soil for several years, and 150,000 per acre were recovered in February 1951 from a field which had not had a wheat crop since 1944. At the same time 50,000 *Contarinia tritici* Kirby cocoons per acre were found in a field which was last under wheat in 1947.

Viable pupae were successfully obtained by soil sampling, and the pupation in the soil and subsequent emergence of the midges can be followed. Thus it is possible also to estimate the numbers of midges that will emerge.

Progress has been made towards predicting outbreaks of these two midges.

INTRODUCTION

Heavy infestations by the wheat-blossom midges, *Contarinia tritici* and *Sitodiplosis mosellana*, occurred in the summer of 1947 in Northumberland and, in view of the paucity of information on such populations other than those at Harpenden, ear counts were made. The method of taking a random sample of 50 ears per field, removing the grain and counting the total number of larvae per ear was tedious and time-consuming. Furthermore, as oviposition continues for over 5 weeks and the larvae remain in the ears for only 4 weeks, the figures represented only the population at the time of sampling.

Soon after the larvae have left the wheat ears and reached the soil, they spin cocoons in which they pass the winter. In the spring, according to Barnes (1941), some at least of the larvae leave the cocoons and wander in the soil for a while. Subsequently, those which are going to emerge as midges that year pupate naked in the soil for 1-3 weeks.

A soil-sampling method was planned to assess the complete larvae population, its distribution and the longevity of the larvae in the field. Barnes (1952) has shown that some *Contarinia tritici* emerge after spending three winters, and some *Sitodiplosis mosellana* after as long as twelve winters in fibre as larvae under insectary conditions.

It was also thought that by recovering pupae from the soil, it would be possible to determine the dates of pupation and so forecast, in any one year, the dates of emergence of the midges. This, together with an assessment of the total number of pupae in the soil in the spring would supply part of the information needed to predict the summer's infestation of the wheat.

METHODS

Samples were taken with a 4 in. wireworm borer to a depth of 6 in. and treated as for wireworm wet extraction (Cockbill, Henderson, Ross & Stapley, 1945) with the following modification.

The 'float' was not boiled but washed back on to the large 60-mesh sieve and spread evenly over the mesh by means of a fine spray. Cocoons of the midges were thus easily seen, removed and counted. The two species were separated by colour. One of my colleagues, R. N. Swanton, had previously devised a method of extracting the larvae from the cocoons by placing the latter in weak potash or soda. This method has been used on samples of all cocoons recovered and has revealed no non-viable larvae.

Tests of efficiency of this method proved that more than 95 % of the cocoons were recovered from soil.

Statistical examinations showed that twenty samples per field gave a sufficiently high degree of accuracy.

OBSERVATIONS ON A SINGLE FIELD

A field in which ear sampling had shown 80 larvae per ear was chosen. The headlands and the areas in midfield, between and beneath the windrows left by the combine harvester, were sampled separately giving the results shown in Table 1.

TABLE 1. *Numbers of cocoons in twenty samples recovered from (a) the headlands, (b) between and (c) beneath the windrows in midfield*

	<i>S. mosellana</i>	<i>C. tritici</i>
(a)	1104	67
(b)	2114	109
(c)	6986	32

This indicates that headland populations are lower than those in midfield. This may be due to soil compaction impeding the burrowing of the larvae into the soil.

The greater population of *S. mosellana* occurring beneath the windrows (c) indicates that on 9 September, at the time of harvesting, only a proportion of larvae had left the ears. Further migration of larvae took place between combining and sampling (17 September). The dry summer of 1947 probably accounted for the considerable number still in the ears at harvest. On the other hand, most of the *Contarinia tritici* had apparently descended by harvest.

Within a week of harvesting some of the straw in the windrows was burned and samples were taken on these sites. A total of 4986 cocoons of both species was

recovered compared with 6986 from the unburned sites. The fact that significantly smaller numbers were recovered from under windrows which had been burned indicates that considerable numbers could be destroyed by burning immediately after harvest. However, it is possible that many remaining in the ears at harvest are parasitized and thus burning might destroy many parasites. Kieffer (1888) considered that *C. tritici* pupae (*sic*) remaining in wheat at harvest were almost invariably parasitized. Dissection of 'late' larvae of *Sitodiplosis mosellana* from different fields has shown degrees of parasitism varying from 25 to 58 %, whilst in larvae which had descended to the soil parasitism ranged from 28 to 40 %. Larvae in the ears at harvest in a dry season may be either parasitized or not because of the lack of opportunity to leave the ears, but larvae in the ears in a wet season, during which there has been ample opportunity for all larvae to leave the ears, may indeed be those that are parasitized.

The samples from the headland and from midfield were taken in halves to depths of 0-3 in. and 3-6 in. Table 2 shows that the majority of the larvae remain in the top 3 in. of the soil but about 20 % were found 3-6 in. down. If the midges are able to emerge successfully from this depth, then normal ploughing would not bury cocoons deeply enough to prevent emergence. Deep ploughing might achieve this, though the larvae leave the cocoons in the spring before pupating and would thus have a chance of adjusting their depth in the soil. Burying the cocoons to a greater depth than normal might have the effect of delaying the emergence of the midges and so getting them out of step with the emergence of the wheat ears. According to Barnes (1941) this does occasionally happen, and when it does the infestation of the wheat is much lower than would have been expected.

TABLE 2. *Numbers of cocoons in twenty samples recovered at 0-3 in. and 3-6 in. from (a) the headlands and (b) in midfield between the windrows*

	0-3 in.		3-6 in.	
	<i>S. mosellana</i>	<i>C. tritici</i>	<i>S. mosellana</i>	<i>C. tritici</i>
(a)	850	58	254	9
(b)	1661	100	453	9

LONGEVITY OF LARVAE IN THE FIELD

The longevity of the larvae was studied on four fields on a farm which suffered a very heavy attack in 1947 and had not carried wheat since. Table 3, which shows the cocoon population and dates of sampling, shows that cocoons remain in the soil for a number of years in the field, thus confirming Barnes's (1952) observations in the insectary.

S. mosellana remains viable longer than *Contarinia tritici*; in field no. 4 even after seven winters 150,000 cocoons per acre of *Sitodiplosis mosellana* were recovered while so far *Contarinia tritici* cocoons have not been recovered more than four winters after a wheat crop. A population of 250,000 cocoons of *C. tritici*

appeared in field no. 3 during 1948, although wheat was not grown in the field. It is possible that this species had arrived from some other field and was breeding on one of its alternate host plants. This seems to be the only explanation of the absence of any of its cocoons in the October 1947 sampling. Table 3 also shows that the percentage reduction of cocoons in the soil is not always regular. The cumulative percentage reductions of *Sitodiplosis mosellana* cocoons per emergence season were 44, 72 and 94 respectively for 1, 2 and 3 years in field no. 1. Similarly, *Contarinia tritici* cocoons decreased by 75 % after one emergence season to 90 % after the second and 98 % after the third. Unfortunately, similar figures for the other three fields are not available, since sampling was not started until several years after the last crop of wheat had been grown.

TABLE 3. *Cocoon populations per acre and dates of sampling*

Field no.	Year of last wheat crop	Month and year of sampling			
		Oct. 1947	Oct. 1948	Feb. 1950	Feb. 1951
<i>S. mosellana</i>					
1	1947	65,150,000	36,300,000	18,850,000	3,900,000
2	1946	13,200,000	3,100,000	3,100,000	1,150,000
3	1945	8,050,000	2,250,000	800,000	650,000
4	1944	650,000	350,000	200,000	150,000
<i>C. tritici</i>					
1	1947	2,000,000	500,000	200,000	50,000
2	1946	450,000	Nil	Nil	Nil
3	1945	Nil	250,000	100,000	Nil
4	1944	Nil	Nil	Nil	Nil

RECOVERY OF PUPAE FROM THE SOIL

In the spring of 1948 the recovery of pupae was attempted by the wet extraction technique with the aim of forecasting the time of emergence of the midges. It was found that viable pupae were easily recoverable.

Samples were taken in 1948 and 1949 from a heavily infested field and stored in tins. The soil was kept moist by periodic spraying with water. The samples were washed at intervals and the number of cocoons, free larvae and pupae recorded. The results given in Table 4 refer only to *Sitodiplosis mosellana* since only one pupa of *Contarinia tritici* was recovered (15 June) and one individual in the process of emerging (7 July).

In 1948 it was possible to record the emergence of the midges. The first *Sitodiplosis mosellana* emerged on 3 July while the peak occurred on 12 July. Fig. 1 shows the percentage pupation and subsequent emergence (in the insectary) during 1948 and the percentage pupation in 1949. This shows the build-up of pupae in the soil and gradual trailing off owing to the emergence of the adults. It also indicates that the first pupae took longer to develop into midges than those that pupated later. The interval between the appearance of the first pupa and the first midge was about

21 days, but that between the peak of pupation and emergence was about 14 days. The last pupation occurred only about 8 days before the appearance of the last midge. Fig. 1 and Table 4 also show the seasonal variations in pupation date.

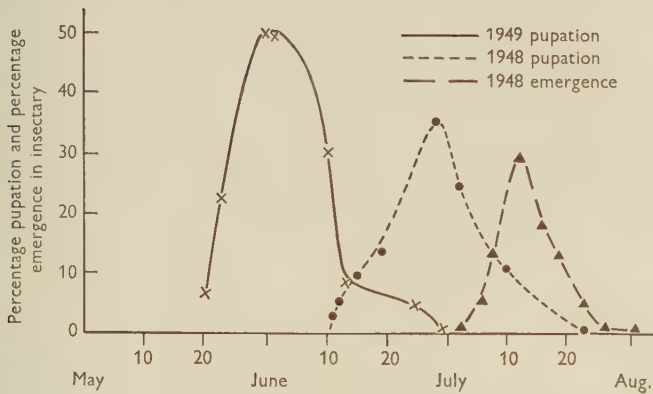


Fig. 1. Pupation of *S. mosellana* in the field during 1948 and 1949, together with its emergence in the insectary.

TABLE 4. *Numbers of cocoons, free larvae and pupae recovered from samples taken on 4 June 1948 and 6 May 1949*

Year	Date	Cocoons	Free larvae	Pupae	Pupation (%)
1948	11 June	28	47	2	2.6
	12 "	1	37	2	5.0
	15 "	101	237	35	9.4
	19 "	23	95	18	13.2
	28 "	14	43	31	35.2
	2 July	25	31	18	24.3
	7 "	29	24	8	13.1
	12 "	8	6	5	26.3
	23 "	5	5	0	Nil
1949	21 May	128	71	14	6.6
	23 "	31	18	14	22.2
	30 "	27	4	31	50.0
	1 June	28	3	30	49.2
	10 "	17	4	9	30.0
	13 "	11	1	1	7.7
	25 "	46	0	2	4.2
	29 "	16	2	0	Nil

The writer is indebted to Dr M. Cohen and Dr H. F. Barnes for encouragement and constructive criticism, to Mr L. C. Crisp, Falloden Mill, Alnwick, Northumberland, for the facilities willingly provided and to members of the Entomology Department for assistance in taking and washing many samples.

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FURTHER OBSERVATIONS ON THE BIOLOGY OF THE CABBAGE ROOT FLY, *ERIOISCHIA BRASSICAE* BChÉ

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In 1951, eggs of *Erioischia brassicae* were first found in the field on 2 May, and the peak period of egg-laying occurred 19–31 May. This was up to a month later than in the period 1948–50.

The periodic removal and examination of the surface soil showed that eggs of *E. brassicae* were continuously present on the host plants from mid-June to early November. Plants under observation during this period showed an average of 285 eggs per plant and other plants exposed to attack from July to November showed an average of 162 eggs per plant. Peak periods of egg-laying, as indicated by numbers of eggs per plant per day, occurred in late June and early July, in mid-August and, to a less extent, in the first half of October. The plants showed no increase in the rate of infestation as the season advanced, although *E. brassicae* has a reproductive capacity of about 100 eggs per female and three to four generations a year.

The difference between the observed egg populations and pupal populations indicated that *E. brassicae* had a heavy mortality rate in its immature stages. The condition of puparia showed that the species was subject to a high degree of natural control, a fact for consideration when direct control measures are formulated.

Pupal diapause extending from October 1949 to May 1951 was observed in one specimen of *E. brassicae* and from October 1949 to August 1951 in one specimen of its Hymenopterous parasite, *Trybliographa rapae* Westw.

Tests with tar-oil winter wash showed that at a concentration of 1¼% it killed eggs of *Erioischia brassicae* and repelled gravid females for approximately a week. Laboratory tests with BHC indicated that it had no adverse effects on the eggs but was larvicidal.

Observations on egg-laying by the cabbage root fly, *Erioischia brassicae* Bché (Miles, 1951), were continued in 1951. April and early May were cool and showery and plant growth was greatly retarded. The harvesting of cauliflowers, set out annually in mid-March, did not begin until late June, a month later than in 1949 and 3 weeks later than in 1948 and 1950. Activity of *E. brassicae* was similarly retarded. Eggs were first found on 2 May, 10 days later than in 1950 and 18 days later than in 1948; and the peak of spring egg-laying occurred in the fourth week of May, a month later than in 1948 and 1949. These seasonal differences in the dates of the spring peak of egg-laying show that there is need for more accurate information concerning the effect of weather conditions on the activity of *E. brassicae*.

The data given below were collected while a method of assessing the activity of *E. brassicae* was being developed.

METHOD

Plants under observation were grown on a plot (10 × 8 ft.) that had been waste for many years. When cauliflowers were first set out in March 1951 there was no established complex of *E. brassicae* and its parasites. Conditions were, however, favourable for infestation because untreated brassica crops were continuously present on the adjoining allotments. Rough grass at the base of the dividing fence and the edges of the allotment path provided shelter for the flies, and buildings on the north and east cut off cold winds. Twenty cauliflowers were set out 2 ft. apart each way. Infestation by *E. brassicae* developed on these plants at the same time as that on the main crop of early cauliflowers about 100 yards away.

In early June and early July the original plants were lifted and replaced with new ones set out in intermediate positions, and observations were made on the numbers of eggs laid in the vicinity of individual plants during the summer and autumn. The surface soil to a depth of $\frac{1}{2}$ –1 in. was removed over a radius of about 2 in. from each plant and fresh soil was drawn round to maintain the soil level. At the first examination the soil from three adjoining plants was accidentally combined and thereafter soil from three adjoining plants was taken as the unit.

Eggs of *E. brassicae* and other anthomyiid flies were extracted from the soil by flotation and sieving. Because the intervals between examinations of soil often exceeded the duration of the incubation period empty chorions were counted as eggs.

NUMBERS OF EGGS OF *ERIOISCHIA BRASSICAE*

The eggs found were mainly those of *E. brassicae*, and data in Table 1 refer only to this species. Small numbers of eggs of *Delia cilicrura* were also found at examinations made after hoeing.

As in previous observations (Miles, 1951), eggs were present continuously throughout July and August. Egg-laying continued until the first week of November, and the number of eggs laid in October was considerable. The periods of activity of the summer generations were not sharply defined, but the numbers of eggs per plant per day suggested that an increase in the rate of egg-laying occurred in the last week of June and the first week of July, in the first fortnight of August, and again, in a modified form, in the first fortnight of October.

While counting the eggs it was noticed that many had irregular holes in the sides and ends. Since larvae of *E. brassicae* always rupture the chorion near the micropyle between the two thickened ribs it was thought that the damaged eggs had been devoured by some predatory animal. Records were not kept of the number of eggs destroyed in this way but it was noted (27 July 1951) that of a total of forty-five eggs fourteen were bitten.

RATES OF EGG-LAYING BY SPRING AND SUMMER GENERATIONS

It is not yet possible to compare accurately the rates of egg-laying by spring and summer generations, but data already collected (Miles, 1950) suggest that the rate at the spring peak is higher than at any subsequent period of the year. No counts of eggs per plant were made in spring 1951, but on two occasions in the fourth week of May, the spring peak of egg-laying, over 100 eggs per plant were collected with the tip of a penknife from the soil around individual plants on the observation plot. This suggests that the numbers of eggs per plant were of the same order as that recorded in 1949 when five plants had a mean population of 117 eggs per plant. If 6 days is allowed for the incubation period of *E. brassicae* (Smith (1927) states that it is 4-5 days) 117 eggs per plant indicates a rate of approximately 20 eggs per plant per day in the fourth week of April, a number greatly in excess of those observed during the period June-November (Table 1).

TABLE 1. *Egg-laying by Erioischia brassicae*

Date	No. of eggs/3 adjoining plants			No. eggs/ plant/day
	Group A*	Group B†	Group C‡	
13 June	0	—	—	—
21 "	17	—	—	1§
2 July	164	—	—	5§
9 "	89	4	1	4§
13 "	25	18	7	1
17 "	54	13	5	2
20 "	12	15	2	1
24 "	22	15	20	2
27 "	45	27	17	3
2 Aug.	21	34	31	1
8 "	41	90	25	3
13 "	62	81	59	4
20 "	79	27	18	2
28 "	31	56	31	2
3 Sept.	29	25	4	1
10 "	38	55	51	2
4 Oct.	16	34	12	<1
10 "	28	62	18	2
17 "	40	62	4	2
22 "	19	16	5	1
24 "	3	4	2	<1
27 "	5	11	0	<1
1 Nov.	8	7	1	<1
5 "	8	0	3	<1
8 "	0	1	0	—
Total	856	657	316	—
Av. per plant	285	219	105	—

* Planted 6 June 1951.

† Planted and watered immediately with tar distillate, 5 July 1951.

‡ Plants partly screened by spinach (*Spinacia oleracea*).

§ From group A plants only. || Discoloured empty chorion.

The low rate of egg-laying observed during the summer months supports the view that infestation is less severe in summer than in spring, and that difficulty in establishing such crops as autumn cabbages and cauliflowers and broccoli, which are usually planted in July, is not necessarily associated with attack by root maggots.

A tentative conclusion that can be drawn from the observations is that the population of *E. brassicae*, as measured by eggs per plant per day, does not increase in density in spite of the occurrence of three generations per annum and a reproductive capacity of about 100 eggs per female (Miles, 1951). This point needs further investigation, but evidence that only small numbers reach maturity is given below.

POPULATIONS OF *ERIOISCHIA BRASSICAE* AT THE END OF NOVEMBER

Four plants, which had been set out on the observation plot on 6 June and left undisturbed and untreated, were lifted on 28 November to 3 December with cubes of soil ($8 \times 8 \times 8$ in.) and examined for the presence of *E. brassicae*. All had good roots, but there were signs of insect injury and the main roots showed more or less extensive blackening.

The soil was washed with a gentle stream of water through two 2 mm. sieves one on top of the other, and all floating material was collected for examination. Puparia, complete and incomplete, were retained on the top sieve. No larvae were seen though the sieves were fine enough to catch those of the third instar, and the Salt & Hollick method for complete extraction (1944) used on two of the soil cubes also failed to reveal larvae. The following numbers of puparia were recorded: plant 1, 18; plant 2, 3; plant 3, 3; plant 4, 8; a total of 32.

Although the plants were set out at the same time as those of group A (Table 1), and nos. 1, 2 and 4 were in adjacent positions, it is not claimed that they had the same egg population, i.e. an average of 285 eggs per plant; but in a plot consisting of 4×5 plants great differences in the distribution of eggs were unlikely. It appeared therefore that there was a heavy mortality rate between egg and pupal stages.

The condition of the puparia gave valuable information regarding the history of *E. brassicae* in the soil. Of the thirty-two puparia collected only three were viable and capable of carrying the species through the winter. One puparium contained a dessicated pupa of *E. brassicae*; another (6 mm. long) contained a pupa of a staphylinid parasite (probably *Aleochara bilineata* Gyll.), and a third (4.5 mm. long) contained a Hymenopterous parasite (probably *Trybliographa rapae* Westw.).

Twenty-six puparia were empty. Nine showed that adults of *Erioidischia brassicae* had emerged since all or part of the larval thorax was present and revealed straight lateral fractures or the remains of the puparium showed a regular fracture at the junction of thorax and abdomen. Eleven puparia, of which seven were complete, showed the characteristic irregular exit holes of staphylinid beetles which may be situated dorsally, laterally, ventrally or at either end. This indicated a loss of 37.5 % of puparia through attack by staphylinid beetles, a proportion comparable

to that of 26.9 % observed during summer and autumn by Wadsworth (1915). Four small puparia showed the irregularly bitten edge at the junction of thorax and abdomen which appears characteristic when adults of *Trybliographa rapae* emerge. It was not possible to say what had happened to the remaining two puparia, of which the thorax and anterior part of the abdomen was left, but the unbroken thorax showed that no adults of *Erioischia brassicae* had emerged.

DIAPAUSE IN *ERIOISCHIA BRASSICAE* AND *TRYBLIOGRAPHA RAPAE*

In March 1950, Dr G. D. Morison sent to Prof. H. W. Miles puparia of *Erioischia brassicae* that had been collected at Aberdeen on 10 October 1949, and twenty-nine specimens were kept in an insectary in a phial with clean sand. Some flies and parasites emerged in April and May 1950; a male of *E. brassicae* emerged on 7 May 1951 and a female of *Trybliographa rapae* (ident. G. J. Kerrich) on 3 August 1951. Evidently the conditions inducing a diapause of 21 months in *Erioischia brassicae* also induced a similar diapause in its parasite *Trybliographa rapae*.

TESTS WITH INSECTICIDES

Tar-oil winter wash as an ovicide. During the spring peak of egg-laying (19-31 May 1951) the author tested the properties of commercial tar-oil winter wash which had been used with promising results by Edwards (1935) and Dawson (1949).

On 19 May a cauliflower plant was treated with 100 c.c. of 2½ % concentration. After 2 days thirty-seven eggs were taken from the soil round the plant and kept in a Petri dish with a film of water to trap the larvae. The eggs were kept under observation for 18 days but none hatched. From a control batch of thirty-seven eggs taken at the same time and maintained under the same conditions thirty-four larvae hatched in the course of 7 days.

On 21 May dilute tar-oil was applied to other plants. A batch of twenty-one eggs collected from a plant 4 hr. after treatment with 100 c.c. of 2½ % concentration showed no hatching in 18 days. On 30 May, 9 days after treatment, thirty-four eggs were collected from a plant treated with 100 c.c. of 2½ % concentration, thirty-eight from one treated with 200 c.c. of 1¼ % concentration, and thirty-four eggs from an untreated plant. The batches of eggs were kept separately in Petri dishes for 9 days, almost twice as long as the incubation period observed by Smith (1927). No eggs hatched after treatment with the 1¼ % concentration; one larva hatched after 5 days from the eggs treated with 2½ % concentration but this egg was probably laid about a week after the plant was treated. Thirty of the thirty-four eggs from the untreated plant hatched in the course of 6 days.

The smell of tar-oil remained in the soil for at least a month after its application, but the attraction of the host plant for gravid females of *Erioischia brassicae* soon outweighed any repellent effect of the tar-oil treatment.

In the observations recorded above, it is shown that of seventy-two (34 + 38) eggs collected 9 days after the host plants were treated with dilute tar-oil only one larva hatched. A further batch of forty-four eggs from a plant which had been treated 9 days earlier gave the following numbers of larvae: 4th day, 4; 5th day, 8; 7th day (not examined on 6th day), 14; a total of 26. The remaining eighteen eggs failed to hatch. If an incubation period of 6 days is allowed (see p. 387) it would appear that eggs laid 1 week or more after the host plants have been treated with dilute tar-oil are not affected by it.

These data may be interpreted in two ways: that the tar-oil kills eggs already laid on the plants and prevents further egg-laying for about a week, or that it is not repellent but is ovicidal for about a week. It is thought that the first alternative is the correct one, for Table 1 shows that plants in groups B and C, which were treated with dilute tar-oil at planting, had significantly fewer eggs on 9 July, 3 days after treatment, than the plants in group A which had not been treated.

Benzene hexachloride. It has already been shown (Miles, 1950) that under certain conditions benzene hexachloride gave to brussels sprouts the same degree of protection from attack by *E. brassicae* as that afforded by calomel dust. Further tests in 1951 showed that BHC was larvicidal but not ovicidal to *E. brassicae*.

DISCUSSION

This limited study indicates the need for more detailed biological and ecological information on *E. brassicae* in order to obtain satisfactory results from ovicidal and larvicidal treatments. The observations on numbers of eggs laid by the summer and autumn generations and on the numbers and condition of puparia at attacked plants at the end of the season suggest that natural factors are effective in limiting the population. The implication that the population of *E. brassicae* does not increase during the annual cycle is far-reaching and affects especially the choice of the control measures which are undoubtedly necessary for the protection of certain crops. The possible effects of insecticides on natural control should be taken into consideration lest disturbance of the faunal complex of which *E. brassicae* is a member may result in conditions in which susceptible crops require continuous protection from April to October instead of only in April-May.

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THE ACTION OF METALDEHYDE ON THE SLUG *AGRIOLIMAX RETICULATUS* (MÜLLER)

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(With 1 Text-figure)

Metaldehyde both as powder and in solution can act on slugs either by contact or as a 'stomach poison'. The characteristic effects of metaldehyde poisoning were immobilization broken by outbursts of unco-ordinated muscular activity and sliming which usually resulted in severe water loss. 24 hr. after treatment with moderate doses slugs were still abnormal and rarely fed within 30 hr. of treatment. It was not possible to determine the M.L.D. with the methods used, but 0.06 mg. solid metaldehyde taken orally could be lethal to slugs of 400–800 mg. body weight. Lethal effects were produced by contact of 1 hr. with concentrations equivalent to 0.0063 mg./cm.². Toxicity increased with rise in temperature and recovery from moderate doses was dependent on slugs being in a saturated or almost saturated atmosphere. No obvious gut lesions were found in slugs which had been dosed with or had eaten metaldehyde. Its action was not by depolymerization in the gut or body cavity. In the light of laboratory and small scale field trials it is suggested that broadcasting and spraying are the best methods of applying the material.

Since the discovery of the slug-killing properties of metaldehyde some twenty years ago much attention has been given to its use on a field scale (Barnes & Weil, 1942; Thomas, 1948). In the absence of adequate information on its mode of entry and method of action it is not possible to say whether the most effective use of metaldehyde is at present being made. The present investigation was therefore undertaken to study in detail the uptake of the material, its physiological action and the effect of changes in temperature and humidity on its action.

METHODS AND MATERIALS

Both culture-reared and freshly captured wild specimens of *Agriolimax reticulatus* (Müller) were used. These did not differ in their reactions to metaldehyde and the bulk of the experiments were carried out on 'wild' slugs. Unless stated to the contrary, all slugs were starved in a saturated atmosphere for 24 hr. before being used in an experiment and in practically all experiments each slug was kept in isolation in a Petri dish lined with moist filter-paper. Weight records were kept during many experiments.

In some experiments, slugs were anaesthetized simply by placing them in narrow 100 ml. beakers containing about 1 ml. of water, into which a slow stream of carbon dioxide was passed. Slugs could withstand this treatment for at least 1 hr. and even

after 5 hr. continuous anaesthetization many slugs recovered without showing any abnormal effects. Different batches of slugs varied in their ability to withstand the effects of carbon dioxide. Sliming occurred during anaesthetization, and this often resulted in a large weight loss (see Table 1).

TABLE 1. *Weight loss induced by carbon dioxide treatment (15–18° C.)*

Slug	Weight before CO ₂ treatment (mg.)	Weight loss after 1 hr. CO ₂ treat- ment (%)	Weight loss after 24 hr. recovery at 100% R.H. (%)
1	835	26.6	13.4
2	933	27.1	24.8
3	717	23.1	17.7
4	795	25.8	24.8
5	703	20.3	1.4
6	585	15.7	3.4

Solutions of metaldehyde were prepared by mechanically stirring an excess of finely powdered metaldehyde with distilled water for 24 hr. and filtering. Analysis showed that the filtrate contained only metaldehyde with no free acetaldehyde, the solubility being 0.018 % at 0.5° C.; 0.020 % at 17° C.; 0.026 % at 30° C. Neutral or alkaline solutions were very stable, and even after 6 months did not contain detectable amounts of acetaldehyde. For use in field trials where mechanical stirring is impracticable a sufficiently concentrated solution can be made by stirring a water suspension at intervals and allowing to stand overnight. For small-scale use, a toxic solution can be prepared by hanging a muslin bag containing coarsely broken 'Meta' tablets in a watering can.

Whilst A.R. metaldehyde was used for practically all experiments some trials were made using the soil fuel 'Meta'. There were no obvious differences in the actions of these two materials.

MODE OF ENTRY OF METALDEHYDE

The literature (see Barnes & Weil, 1942; Thomas, 1948) suggests that metaldehyde acts as a contact poison. The experimental evidence supporting this conclusion is scanty, and much of it open to the criticism that adequate precautions were not taken to prevent treated slugs from eating traces of metaldehyde. Jary & Austin (1937) reported that sliming occurred when minute quantities of metaldehyde were placed on slugs and that five out of fourteen treated slugs were dead on the day following treatment. Thomas (1948) described experiments made to check the statement of Persing (1944) that metaldehyde had a contact action on the snail *Helix aspersa*. According to Thomas, metaldehyde placed on the bodies of healthy snails '... is merely sloughed off with the mucus as is any inert dry powder such as finely divided limestone'. He concluded, therefore, that metaldehyde had no contact action on snails. Similar experiments on slugs are not described but Thomas appears to assume that metaldehyde must be eaten to produce a lethal

effect. He does state, however, that the irritant effect (sliming) might possibly be due to some contact action either by metaldehyde or dry bran on slugs partially poisoned by metaldehyde.

To elucidate the mode of entry of metaldehyde the following tests were made to determine its fumigant, contact and 'stomach' action on *Agriolimax reticulatus*.

(1) *Fumigant action*

Twenty slugs were confined over metaldehyde in a saturated atmosphere, the perforated floor of the testing chamber being 2 cm. above the layer of metaldehyde. Twenty control animals were exposed over calcium carbonate. Even after 3 days' exposure the slugs were not obviously affected by metaldehyde and readily fed when offered lettuce, indicating that metaldehyde has no important fumigant action.

(2) *Contact action of powdered metaldehyde*

Powdered metaldehyde was placed on the backs of slugs, and control animals were treated in a similar way either with calcium sulphate or carbonate. The slugs were kept in a saturated atmosphere under constant observation with every precaution to ensure that the material was not eaten. Irrespective of the site of treatment the metaldehyde-treated *A. reticulatus* showed the following reactions at 15–17° C. Some 15–100 min. after application locomotion ceased, tentacles were withdrawn and constrictions appeared spasmodically over the head and body. During the ensuing period of immobilization which might last several hours, occasional outbursts of locomotor activity would occur but they lacked co-ordination. When stimulated with forceps, treated slugs would attempt to move, but since movements were not co-ordinated the animals remained on the same spot. Sliming always occurred. In some, a thick chalky slime was produced, but in the majority the slime was thin and practically colourless. The slime had a wetting action on metaldehyde. Although the slugs were immobilized, sliming always continued until the metaldehyde was sloughed off. Removal of the metaldehyde rarely took less than 30 min. but never more than 4 hr. Twenty-four hours after treatment slugs were never normal and rarely fed within the succeeding 24 hr. In some cases the metaldehyde-treated slugs died as a result of the treatment.

Slugs treated with calcium sulphate and carbonate were quite normal 24 hr. after treatment and in many cases fragments of chalk still adhered to the epidermis. There was no noticeable effect on activity and no sustained sliming to remove the material.

Under field conditions the foot of the slug would be the most likely surface to come into contact with metaldehyde. It proved difficult to keep the substance in contact with the foot for periods greater than 5 min. but even with such short periods of contact the effects recorded above were noted.

Experiments were also made on slugs under carbon dioxide anaesthesia to ensure that metaldehyde was not eaten during the experimental period. At the end of the

treatment any powder remaining on the slugs was scraped or washed off and the slugs left in individual Petri dishes to recover from the anaesthetic. All metaldehyde-treated slugs showed toxic symptoms and a varying number died. The results of a typical experiment are shown in Table 2.

TABLE 2. *Contact action of powdered metaldehyde on anaesthetized Agriolimax reticulatus (15–17° C.)*

Length of treatment (hr.)	Condition of slugs 24 hr. after treatment					
	Powdered metaldehyde			Powdered calcium carbonate		
	Normal	Moribund	Dead	Normal	Moribund	Dead
1	13	2	3	16	1	1
3	8	2	8	18	0	0

(3) *Contact action of metaldehyde solution*

Even though chemical handbooks state that metaldehyde is insoluble in water, it was noticed that water which had been in contact with metaldehyde was irritant to slugs. Slugs were painted with drops of saturated metaldehyde solution coloured with methylene blue. Thus the path of a solution applied to the epidermis could be traced, establishing with certainty whether or not the mouth region of the slug became contaminated with metaldehyde. Trials showed that, providing the slug was held vertical with its head uppermost, then the back and tail portions could be flooded with liquid without contaminating the anterior foot or mouth regions. Control slugs were treated with distilled water coloured with methylene blue.

One to eight minutes after dabbing with saturated metaldehyde solution slugs became immobilized and secreted thick white slime. Abnormal behaviour lasted for 10–24 hr. and many slugs were killed by the treatment.

The contact effect was studied quantitatively by using filter-papers (9 cm. diameter) placed in Petri dishes and flooded with 2 ml. of 0.02 % metaldehyde solution (0.0063 mg./cm.²). Single slugs were placed in such Petri dishes and left in contact with the solution for varying periods of time. Some experiments were made using filter-papers impregnated with metaldehyde from chloroform solutions over the range 0.002–0.2 %. The chloroform papers were thoroughly air-dried and then flooded with 1 ml. water before use; papers thus impregnated gave essentially the same results as those treated with aqueous solutions. The contact effect was also demonstrated by placing individual slugs in small rectangular cells in a paraffin block into which known amounts of aqueous solution could be introduced.

Slugs placed so that only the foot was in contact with the impregnated filter-paper quickly showed signs of metaldehyde poisoning. The various symptoms observed when solid metaldehyde was used were recorded but their onset was more rapid and their intensity greater. Within 5 min. of being placed on the paper, tentacles were withdrawn and locomotion ceased. Thick chalky slime would always appear 25–60 min. after contact, whereas when solid metaldehyde was used this

type of sliming did not always occur. Results obtained from typical experiments with 0.02 % aqueous solution and using anaesthetized and unanaesthetized slugs are shown in Table 3. Contact with 0.002 % for 3 hr. produced up to 10 % deaths. Two other effects, immobilization and inhibition of feeding, are also important factors in the value of metaldehyde for slug control. Exposures as brief as 5 min. were sufficient to produce immobilization which, depending on the temperature conditions, lasted for 10–24 hr. (see p. 401). After contact with 0.002 % solution for intervals of 15 min. to 3 hr. some 50 % were able to feed within 24 hr. of treatment. Using 0.02 % marked differences in feeding ability were noted, depending on the length of treatment. Neglecting those slugs killed by the treatment, 50 % were able to feed within 2 days after 15 min. treatment and within 3 days after 1 and 3 hr. contact.

TABLE 3. *Contact action of metaldehyde solution on Agriolimax reticulatus*

Length of treatment (hr.)	Metaldehyde solution			Controls (distilled water)		
	Normal	Moribund	Dead	Normal	Moribund	Dead
(a) Anaesthetized slugs (16–19° C.)						
1	6	3	3	12	0	0
3	4	3	11	13	2	3
5	0	3	18	10	3	8
(b) Unanaesthetized slugs (18–22° C.)						
1	10	0	2	12	0	0
3	1	0	11	12	0	0

TABLE 4. *Weight losses (mg.) induced by contact with metaldehyde solution*

Length of treatment (hr.)	Metaldehyde solution		Controls (distilled water)	
	After treatment	After 24 hr.	After treatment	After 24 hr.
1	–21.99 ± 3.52	–24.29 ± 6.77	–1.98 ± 4.24	–4.66 ± 3.77
3	–26.93 ± 5.24	–32.6 ± 6.68	+8.3 ± 6.6	+6.75 ± 4.41

After exposure to filter-paper impregnated with 0.02 % metaldehyde solution, slugs were placed in Petri dishes containing moist filter-papers.

Even though slugs were maintained at 100 % R.H. during and after contact treatment, weight losses of 25–35 % were recorded, chiefly water loss (see Table 4). Water loss via sliming and water uptake could occur simultaneously: for example, slugs dehydrated for 5 hr. (weight loss 25–50 %) would slime copiously on metaldehyde-treated filter-papers, but after 1 hr. contact they usually showed weight increases of up to 10 %. Controls similarly dehydrated showed gains of 25–50 % in 1 hr. when placed on filter-papers moistened with distilled water.

These experiments fully support the observations of Jary & Austin (1937). Metaldehyde can exert a contact effect as a solid or in solution, and if contact is sufficiently prolonged its action may be lethal.

*(4) Metaldehyde as an internal poison**(a) Metaldehyde solution*

Metaldehyde solutions were injected into the crop via the mouth using an 'Aglā' micro-injection syringe fitted with a glass capillary tube shaped to pass through the buccal mass and down the slender oesophagus. The delivery end of the tube was bulb-shaped to prevent laceration of the tissues and the escape of liquids from crop to mouth during the course of injection. To ensure that slugs in which regurgitation occurred were not included in the assessment of results, solutions were coloured with methylene blue.

The amount of solution which could be passed into the crop without regurgitation was limited to 0.1–0.2 ml. for *A. reticulatus* weighing 300–800 mg. In only a few cases, out of many trials, was 0.2 ml. injected without some regurgitation occurring.

Quantities as small as 0.01 ml. ($\equiv 0.002$ mg.) induced symptoms of metaldehyde poisoning, and these were very marked with the maximum amount (0.1 ml. $\equiv 0.02$ mg.). The various effects described for the contact experiments were observed in injected slugs but they were slower to appear and, apart from copious white sliming, were never so intense. At 20° C., using the maximum dose (0.1 ml.) 28 % (approx.), mortality was recorded within 3 days of injection (Table 6). Injected slugs did not feed in the 36 hr. period following injection.

(b) Metaldehyde powder

Methods. Micro-injection guns were used to force known amounts of dry materials such as powdered metaldehyde into the buccal cavity and crop of slugs. These were constructed either from hypodermic needles with the ends cut off or from fine-bore stainless steel tubing. The guns had tight-fitting plungers made from pivot steel and were loaded by pressing the delivery end into a small heap of finely powdered test material, the plunger being sufficiently withdrawn to allow the substance to be packed into the shaft. After this preliminary loading the shaft was pressed until the loading mark was just visible above the tube. When a high degree of accuracy was necessary this operation was carried out under a binocular-dissecting microscope. This movement, if the loading had been effective, resulted in a small cylinder of test material protruding from the delivery end. With a steady pressure on the plunger, the delivery end was again pressed into a small heap of test substance, ensuring that the end of the tube was uniformly packed. Surplus material was carefully wiped off. Each gun was calibrated by filling the instrument and then weighing the amount ejected. In the guns of very fine bore (0.18 mm.) graduations were not necessary for it was only possible, using the procedure described above, to pack a small length of the column with powder.

The accuracy of the method was low. Whilst different guns, when carefully

loaded, were capable of ejecting the following amounts of metaldehyde: 0.32 ± 0.1 , 0.24 ± 0.1 and 0.06 ± 0.04 mg., it was not possible to make any allowance for errors introduced in the course of injecting metaldehyde into test animals. Particles adhered to the guns to a varying extent after withdrawal and using very small amounts (< 0.1 mg.) it was never possible to determine whether all or part of the injected material had been regurgitated.

In order to obtain concentrations of metaldehyde below the content of the smallest gun used (0.06 mg.) the substance was diluted with either glucose or calcium sulphate or carbonate. Comparative trials failed to show any specific effect of the diluent on the activity of the metaldehyde.

Both anaesthetized and non-anaesthetized slugs were used in the experiments. In most experiments slugs were dissected to determine whether the metaldehyde had remained in the animal and whether any injuries had been caused by the injection. The death-rate from the injection procedure was negligible.

Results. As might be expected, slugs reacted more slowly to the presence of solid metaldehyde than to dissolved metaldehyde. Results of different experiments carried out under standard conditions varied over a wide range, and it was evident that the experimental technique was too crude and the reactions of the animals too variable to permit an accurate assessment of the median lethal dose. In Table 5 percentages are given based on a number of different experiments. In view of the many sources of error, known and unknown, it is clear that the figures represent no more than the trends shown with different dosage rates. Death from metaldehyde poisoning was long drawn out. As Table 5 shows, even at high dosage rates deaths were still occurring 3 days after treatment and the greater part of the material could be recovered from the gut after death. In a few cases metaldehyde was actually defaecated.

TABLE 5. *Lethal action of powdered metaldehyde injected into the crop of Agriolimax reticulatus*

Amount injected (mg.)	No. of slugs	Percentage kill Days after injection		
		1	2	3
0.003	30	0	0	0
0.01	40	5	10	15
0.03	40	22	30	35
0.06	80	30	56	66
0.22	30	63	87	—

No deaths were recorded in the controls which were injected with calcium sulphate. All experiments were carried out at 20°C .

From these investigations on the mode of entry of metaldehyde it is clear that the material can exert its effects both by contact and as an internal poison.

MODE OF ACTION OF METALDEHYDE

(1) *Injection of solutions into the haemocoel*

Since slugs reacted to internal doses less rapidly than to external treatment, it would seem that the material was not rapidly transported from the gut. The effect of injecting metaldehyde solution directly into the haemocoel was therefore studied.

Methods. An 'Agla' syringe fitted with an ordinary fine injection needle was used. In preliminary trials to test the influence of site of injection on sensitivity to metaldehyde, batches of slugs were injected either on the dorsal, lateral or ventral surfaces at one of the following positions: anterior to the mantle; posterior to the mantle; tail region. The nine injection sites were compared and the effects produced were found to be similar. In the detailed investigations therefore, injections were made in the middle region of the foot. Solutions were coloured with methylene blue, and even unanaesthetized slugs could be injected with amounts up to 0.15 ml. with little loss of fluid. In all experiments equal numbers of control slugs were injected with water coloured with methylene blue.

Results. Injection of 0.1 ml. metaldehyde directly into the haemocoel produced an immediate reaction. Usually before the whole amount had been injected the slug was completely immobilized and thick white slime was copiously produced. Even amounts as small as 0.01–0.02 ml. (0.002–0.004 mg.) produced this immediate reaction. From Table 6 it is evident that the lethal effect was subject to greater variation than that shown in oral injections and contact treatment. On the whole, slugs recovered more rapidly from haemocoel injections than from contact treatment or crop injections.

TABLE 6. *Comparison of the lethal action of oral injection, haemocoel injection and contact treatment (20° C.)*

Oral injection, 0.1 ml.	Haemocoel injection, 0.1 ml.	Contact with 0.1 ml. for 3 hr.
8.75 ± 1.7	6.75 ± 3.13	10.37 ± 1.77

These means refer to the deaths recorded per twenty-five slugs 3 days after treatment. They are based on nine experiments in each of which twenty-five slugs were treated in each group. Contact treatment was carried out in paraffin-wax cells (see p. 395). The maximum death-rate in controls treated with water was three per twenty-five slugs.

Control slugs injected with equivalent amounts of water were not immobilized by the treatment and showed normal activity and feeding immediately after injection. Some white slime was usually produced at the time of injection, but the animals in spite of the shock-effect of the treatment always showed a gain in weight, whereas those given metaldehyde showed a marked weight loss (Table 7).

TABLE 7. *Weight changes (mg.) produced by injections into the haemocoel*

Metaldehyde solution (100 mg.)		Controls (distilled water 100 mg.)	
1 hr. after treatment	24 hr. after treatment	1 hr. after treatment	24 hr. after treatment
-66 ± 31.4	-78 ± 29.0	± 83.5 ± 34.7	+58.3 ± 31.4

(2) *The action of acetaldehyde and paraldehyde*

The effects of acetaldehyde, paraldehyde and metaldehyde were compared with the aim of elucidating the mode of action of metaldehyde.

Contact action was tested by the normal filter-paper technique. At both 15 and 20° C. slugs were quite normal even after 3 hr. contact with 2.0 % solutions of either acetaldehyde or paraldehyde, whereas 0.02 % metaldehyde produced the toxic effects already described. Further, the two former solutions did not produce toxic symptoms when injected orally or directly into the haemocoel. Whilst some immobilization was shown, slugs recovered sufficiently to move about and eat within 24 hr. of treatment.

These observations show that the toxic effects of metaldehyde are not due to the formation of acetaldehyde and are not equivalent to the action of paraldehyde. The fact that acetaldehyde is without toxic action even at concentrations 100 times greater than that of the usual metaldehyde solution would suggest that any depolymerization occurring in the gut or blood of slugs would result in a lessening of the toxic action of the material.

(3) *Histological effects of metaldehyde*

Thomas (1948) states that the gut wall becomes transparent as a result of metaldehyde poisoning, and when this condition is reached the slug will eventually die. To determine the extent of this change slugs killed by metaldehyde and an equal number poisoned by carbon dioxide were dissected. These observations showed no transparency in the metaldehyde-treated slugs which was not of normal post-mortem origin. Transparency results from a sloughing off of the thin mucous membrane of the crop and this occurred whatever the cause of death, for it was noted both in culture slugs and in field slugs which were found dead. Further, metaldehyde does not accelerate the removal of the mucous membrane. Crops excised from healthy slugs were ligatured at both ends after having small amounts of metaldehyde pushed into them and kept in *Helix* Ringer (Pantin, 1946) along with similarly prepared controls containing equivalent amounts of calcium carbonate or calcium sulphate. Under such conditions as far as macroscopic inspection showed, the membranes remained healthy up to 24 hr.

(4) *Effect of temperature and humidity*

Slugs are without a waterproofing layer, and in an unsaturated atmosphere lose water passively to the environment (Howes & Wells, 1934). Death from desiccation can take place rapidly; for instance at 70 % R.H. (15° C.) slugs will die from desiccation in 7–17 hr. It is obvious, therefore, that if slugs suffer from metaldehyde poisoning and are immobilized, their loss of water due to sliming coupled with exposure to low humidities will produce death. This was verified in the course of the investigations. It was also shown that metaldehyde-treated slugs exposed to

saturated atmospheres were not able to regain their water content at the same rate as untreated slugs.

High temperature enhances the toxicity of metaldehyde (see Table 8). Detailed studies on 'contact'-treated slugs showed that temperature influenced both uptake and toxicity. Thus, slugs placed in contact with metaldehyde at 15° C. for 3 hr., and then kept at 20° C. for 21 hr. showed a lower death-rate than slugs kept at 20° C. throughout the experiment or kept at 20° C. for treatment followed by 21 hr. at 15° C. This temperature effect is certainly of practical importance, and it helps to explain one of the findings of Thomas (1948) who concluded on the basis of field trials that wet warm nights followed by dry warm days gave the highest catches and highest mortalities. It is now clear that warmth whilst influencing the activity of slugs also increases the toxic action of metaldehyde.

TABLE 8. *Effect of temperature on the toxic action of metaldehyde*

Quantity of metaldehyde (ml.)	Exposure (hr.)	No. of slugs used at each temperature	Deaths 24 hr. after treatment	
			15° C.	20° C.
0.1	1	25	1	6
0.1	3	30	0	11
0.2	1	35	1	7
0.2	3	70	7	36

FIELD TRIALS WITH METALDEHYDE SOLUTION

To assess the toxicity of metaldehyde solutions to slugs under field conditions the following experiments were carried out.

(1) *Field exposure of laboratory-treated slugs*

Slugs were placed in contact with metaldehyde solution using paraffin block cavities just large enough to contain single slugs and up to 0.2 ml. solution. Slugs were left in contact with the solution for 5 min., 15 min., or 3 hr., then removed and the surplus liquid absorbed by filter-paper. Treated slugs were then placed in the garden on bare rough soil under a coarse mesh wire-netting cage to exclude birds. Control slugs were treated with water and also placed out of doors under wire-netting cages. Field exposure began in the late afternoon (16 hr., G.M.T. approx.) and counts were made the following morning to determine the number killed and the number which had been able to seek shelter.

It is evident from Table 9 that even 5 min. contact with the solution was sufficient to produce a degree of immobilization lasting 10–24 hr. under field conditions. Furthermore, given warm, dry conditions a high mortality was recorded with such a treatment.

(2) *Container experiments*

In these experiments galvanized iron containers 60 cm. long and 26 cm. diameter with bottoms and tight-fitting lids of perforated tin were filled with soil to within 10–15 cm. of the brim and sunk in the ground so that the outside and inside soil

levels were approximately the same. The soil surface within the container was slightly compacted and made relatively smooth.

Metaldehyde solution (0.02 %) was applied to the soil surface within the containers to give the following amounts in mg./m.² of metaldehyde: 75, 226, 376, 488 (equivalent to 20, 60, 100 and 130 ml. of solution per container). Controls were similarly sprayed with 20 ml. water. (In laboratory experiments with impregnated filter-papers toxic effects were obtained with 2 ml. per filter-paper which is equivalent to 63 mg./m.².)

TABLE 9. *Effect of field conditions on laboratory-treated slugs*

Date, 1951	Period of contact and temperature	No. on soil after 24 hr.		No. killed		Weather
		Treated	Controls	Treated	Controls	
12 March	3 hr. (20° C.)	10	2	0	0	Snow
20 March	3 hr. (20° C.)	9	9	2	0	Heavy frost
5 April	3 hr. (20° C.)	9	10	9	2	Windy; very dry
6 April	3 hr. (20° C.)	9	0	9	0	Dry
10 April	15 min. (15° C.)	9	0	0	0	Frost
12 April	15 min. (15° C.)	10	1	0	1	Windy; dry
26 July	15 min. (18° C.)	10	2	9	1	Warm; showery
26 July	15 min. (18° C.)	8	0	8	0	Warm; showery
9 April	5 min. (15° C.)	6	0	6	0	Warm; dry
10 April	5 min. (15° C.)	9	0	0	0	Frost
12 April	5 min. (15° C.)	10	1	10	1	Wind; dry
26 July	5 min. (18° C.)	5	—	5	—	Warm; showery
26 July	5 min. (18° C.)	10	—	8	—	Warm; showery

In each experiment ten treated and ten control slugs were placed under field conditions. Columns 3 and 4 show the number of slugs found on the soil surface with little or no shelter. In the case of metaldehyde-treated slugs 80 % were discovered within a radius of 25 cm. of the site of deposition, whereas many control slugs had travelled over 1 m.

The soil in the containers was sprayed with the appropriate solution at approx. noon (G.M.T.), then 2½–3 hr. later, ten *A. reticulatus* together with unsprayed lettuce were placed in the container. The condition of the slugs was recorded at approximately 24 hr. intervals over a period of 3–5 days.

Results. The total deaths recorded in typical experiments, together with temperature and humidity data, are shown in Fig. 1. Under warm dry conditions mortality was 100 % at all concentrations, although the control slugs laid eggs and fed quite normally under the same physical conditions. Cool and wet weather at the beginning of an experiment decreased total mortality (Expts. 3 and 5), but even under such conditions 100 % mortality still occurred in the cylinders sprayed with 488 mg./m.².

Observations made on slugs at various times of the day confirmed the known effects of humidity and temperature on metaldehyde-treated slugs. On warm days many slugs which appeared normal or only slightly abnormal in the morning would be moribund or dead in the afternoon. On the other hand, slugs which appeared

to be moribund in the afternoon would recover if overnight conditions were cool and wet. Feeding is greatly affected by metaldehyde treatment. Although some feeding was recorded at all except the highest concentrations it was never so extensive as that shown by the controls.

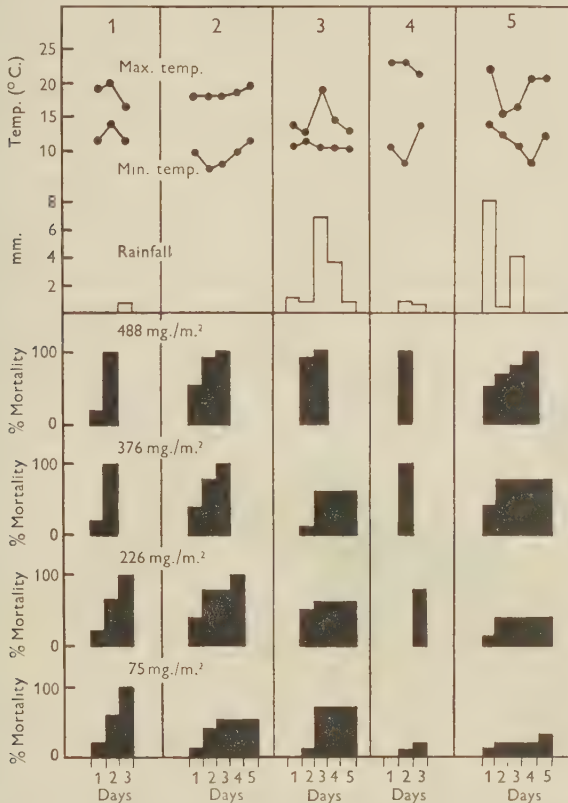


Fig. 1. Histograms of five experiments to show the lethal effect of different amounts of metaldehyde solution under field conditions. The amount of metaldehyde solution is expressed as milligrams of metaldehyde per square metre.

Even though conditions within the tins were not equivalent to normal field conditions the experiments demonstrated that metaldehyde solutions were toxic when applied to soil.

(3) Experiments using large slug-proof enclosures

To simulate natural conditions as far as possible, and yet be able to make counts on known samples of slugs, a slug-proof enclosure, 100 cm. long $\times$ 90 cm. deep was made out of phosphor-bronze gauze (1 mm. mesh). At the corners, uprights of 2 cm. diameter conduit piping were firmly wired to the outside of the gauze. Around

the upper edge an iron frame 0.3 cm. thick $\times$ 2.5 cm. deep was soldered to the outside. The lid of phosphor-bronze, with its edges also strengthened by an iron frame, fitted over the box, and to ensure a 'slug-proof' condition the inner edge of the lid was lined with strip rubber of the 'draught-excluder' type. This bottomless box was sunk into the ground to a depth of 45 cm., thus leaving an extensive air space above soil level.

Three types of experiment were made, using saturated metaldehyde solution in all at the rate of 500 mg./m.² (2½ l.) and an equivalent amount of water for the controls. After each experiment the contaminated soil was removed to a depth of 20 cm. and replaced with fresh soil.

Series I. The soil surface was sprayed approx. at noon (G.M.T.). Three to four hours later, four white tiles (15 $\times$ 15 cm.) to provide cover, fifty *A. reticulatus* and a few unsprayed lettuce leaves were placed on the soil. These experiments were essentially of the same type as those described as 'container experiments'.

Series II. Slugs were placed in the pen along with tiles and lettuce at 11 hr. (G.M.T.) and allowed to seek shelter. When the contents of the pen were sprayed 3-4 hr. later, no slugs were visible.

Series III. In these experiments the pen was not sprayed, but lettuce was soaked in the solution and then placed in an otherwise metaldehyde-free pen in which fifty slugs had already been placed and taken shelter. The treated lettuce was removed after 24 hr. and replaced by fresh (unsprayed) lettuce.

TABLE 10. *Effect of metaldehyde solution under field conditions*

No. of exp.	Duration (days)	Max. temp. (range ° C.)	Rainfall (mm.)	Occurrence of feeding	Slugs found dead (%)
Series I. Slugs placed on sprayed soil					
1	4	12-15	10.3	None	68
2	2	22-24	0	None	96
3	4	15-23	12.5	None	96
Series II. Slugs under cover at time of spraying					
1	5	13-21	10.4	Moderate	28
2	4	17-23	0.7	Slight	92
3	3	15-24	1.1	Slight	96
Series III. Only food material sprayed					
1	3	12-21	2.8	Moderate	36
2	4	15-18	4.5	Moderate	10
3	5	11-20	1.4	Moderate	0

Fifty slugs were used in each experiment. Extensive feeding occurred in all control batches and no deaths were recorded.

From Table 10 it is clear that metaldehyde solution can exert a lethal effect under field conditions, the best results being achieved where the material was applied generally to the surfaces likely to be traversed by slugs. In Series III, where slugs

were exposed to sprayed food material for 1 day, the lethal effect was small in spite of the fact that the treated lettuce was eaten extensively. The daily counts did show, however, that a majority of slugs remained immobilized on the soil surface for some period during the first 2 days. Given dry warm conditions the death-rate would no doubt have been greater.

In general, these experiments emphasize the value of the contact action of metaldehyde, at the same time indicating its non-persistent nature and its dependence on suitable weather conditions.

DISCUSSION

This investigation proves that metaldehyde can exert a toxic action on *A. reticulatus* either by contact or as a 'stomach' poison; the toxic effects appearing more rapidly in contact treatment. Metaldehyde stimulates water loss via mucus secretion, produces immobilization (the 'anaesthetic effect' of Thomas, 1948) and inhibits feeding. With moderate doses complete recovery may occur providing the slugs are kept in a saturated atmosphere. The recovery rate is greatest at low temperatures. Death from metaldehyde poisoning is not due simply to desiccation, for it can occur even when slugs are kept at 100 % R.H. and when the treatment has induced only a moderate water loss.

The data so far available suggest that metaldehyde is a nerve poison, but how it produces its various effects is not known. Its toxic action is clearly not dependent on depolymerization prior to absorption at the site of action.

Now that two modes of entry are known to occur it should be possible to place the field use of the material on a more rational basis. To make the fullest use of the contact effect spraying or broadcasting would appear to be the simplest method of application.

A saturated solution can bring about the death of slugs under field conditions and it can inhibit feeding. Obviously it is not persistent and would need frequent applications to ensure a high degree of control. Greater concentrations of the material could be obtained by applying it as a suspension. In horticulture it might be applied when non-edible plants were being watered and it should be of value as a preventive spray applied to hedgerow bottoms, in protecting seed-beds and plants cultivated for decoration. At present, in view of the known toxic effects of metaldehyde on man (Lewis, Madel & Drury, 1939) it would not be advisable, without detailed investigation, to use the solution on a large scale on edible crops, although it is unlikely because of its non-persistent nature, that toxic doses would remain on sprayed plants.

At present, solid metaldehyde is usually used in bait form, the bait being spaced about the area in small heaps or as 'biscuits'. According to Barnes & Weil (1942) the efficiency of baiting is dependent upon the presence of a suitable attractant such as bran. Thomas (1948) has shown that his biscuit form has the added advantages that it does not break up very readily in wet weather and that it is more

resistant to attack by moulds. While there is much to be said for attracting slugs to the material when it is used in the form of discrete heaps, it should be possible, relying on chance contact action, to dispense with an attractant when broadcasting metaldehyde. Thomas (1948) clearly showed his 'broken-biscuit' preparation when broadcast to be more effective than the usual bran-metaldehyde mixture. Now that the value of the contact effect has been established, trials should be carried out to determine whether bran in the 'broken-biscuit' preparation can be replaced by a 'non-food' carrier without impairing the efficacy of the material.

The writers wish to acknowledge the help given by Dr W. K. R. Musgrave who determined the solubilities given on p. 393 and tested metaldehyde solutions for the presence of acetaldehyde. The investigation was wholly financed by a grant from the Agricultural Research Council.

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INSECTICIDES FOR PREVENTING BARNACLE SETTLEMENT

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(With Plate 16 and 2 Text-figures)

During the summer of 1950 experiments were made to test the use of insecticides for preventing the fouling of oyster spat collectors by the barnacle *Elminius modestus*.

The insecticides used were DDT, BHC (benzene hexachloride- γ -isomer) and dieldrin (hexachloro-epoxy-octahydro-dimethano-naphthalene), made up as 5% solutions and tested on egg-box collectors and quarry tiles. The tiles were used for frequent observations on settlement.

When the solutions were *sprayed* on to the surfaces, in sufficient quantity to give a covering of 200 mg./ft.² of active insecticide, none of them interfered with either barnacle or oyster settlement.

When the test surfaces were *dipped* in the solutions, DDT and BHC prevented barnacle settlement almost completely, while dieldrin reduced it by approximately 50%. Settlement of *Ostrea edulis* was also reduced on the treated surfaces.

Urea-formaldehyde resins made up as varnishes, to which DDT or BHC had been added, were also tested. A varnish incorporating 20% by weight of DDT provided an effective anti-fouling compound which lasted at least 3 months and completely prevented barnacle settlement.

The possibility of insecticides dissolving into the water and inhibiting barnacle growth on adjacent surfaces is discussed.

Since its introduction (Bishop, 1947; Crisp & Chipperfield, 1948; Stubbings, 1950) *Elminius modestus* Darwin has multiplied with great rapidity and is now the dominant sedentary organism, between tide marks, in the Rivers Roach and Crouch in Essex. It competes effectively with young oyster spat for food and settling space (Knight-Jones, 1948) and must certainly be responsible for destroying vast numbers of free-swimming oyster larvae (Cerruti, 1941). It has seriously affected the use of artificial oyster spat collectors with which experiments are being conducted in Essex. Previously the practice had been to expose such collectors only a few days before the major spatfall, to ensure that settling oyster larvae were presented with surfaces as free from competing organisms as possible (Cole & Knight-Jones, 1949), but *E. modestus* is now so abundant that it smothers clean surfaces within 48 hr., making it difficult for young oysters to become established. The use of insecticides to combat this fouling was considered in view of the results obtained with DDT in America (Loosanoff, 1947; Engle, 1949) and Germany (Kühl, 1948). Loosanoff found that barnacle settlement was inhibited by DDT, while *Gryphaea virginica* settled more abundantly on the less heavily fouled surfaces.

Experiments were planned for the summer of 1950, but owing to an early oyster spatfall and an unavoidable delay in obtaining some of the materials, no conclusive evidence as to their effects on oyster larvae was obtained. The results may, however, be of some use in restricting fouling. The insecticides used were DDT, BHC (benzene hexachloride- γ -isomer) and dieldrin (hexachloro-epoxy-octahydro-dimethano-naphthalene).

MATERIALS AND METHODS

The insecticides were dissolved in a mixture of four parts vaporizing oil to one of benzene at the following strengths: 5 % DDT, 5 % BHC, 5 % dieldrin and 10 % BHC.

The varnishes tested were prepared from urea-formaldehyde resins. It has been found (Block, 1948*a, b*; Bracey, 1950; Anon., 1950) that when DDT is added to such a varnish at the rate of 20 % per dry weight of resin, an effective insecticidal compound is produced. If this is baked at a controlled temperature, a solid solution of DDT in resin is obtained from which the DDT crystallizes out as a white bloom of fine acicular crystals. Crystallization will continue for at least 12 months and can be stimulated by rubbing or other mechanical means. An additional varnish, to which 20 % BHC has been added, was also used.

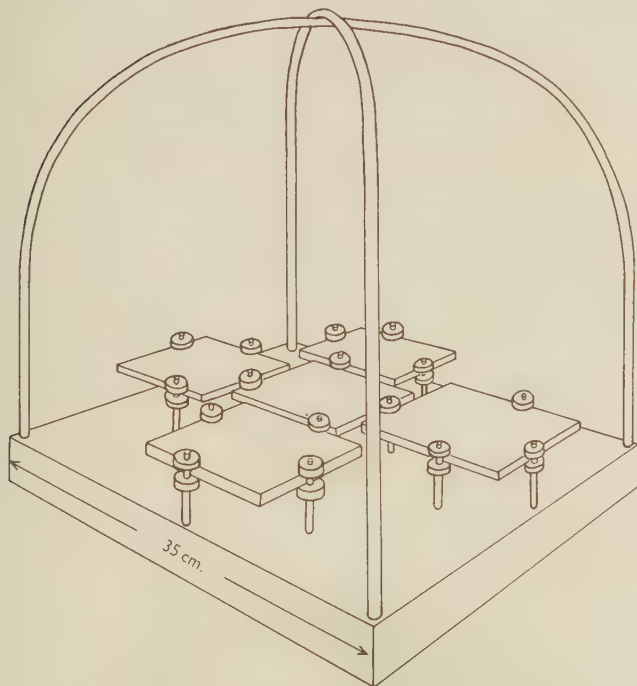
Solutions were tested on egg-box oyster spat collectors (Needler, 1941), and 4 × 4 in. quarry tiles, with one flat and one corrugated side. The varnishes were tested on tiles, on wooden poles which formed a framework carrying the collectors and on plain cardboard egg-box fillers.

The collectors were suspended from frameworks set out at low water (Pl. 16, fig. 1) and were exposed for long periods, in order to provide information on the effect of insecticides on spatfall, while the quarry tiles were examined frequently for settlement of barnacles and spat. The tiles were exposed in sets of five, conveniently fixed to specially made concrete blocks (Text-fig. 1).

The blocks were exposed at low water of equinoctial spring tides (L.W.E.S.T.). Periodically they were lifted, the tiles removed, slipped into a combined holder and counting grid, and counts made of the numbers of settled organisms. After examination, the tiles were replaced on the blocks and returned to the water as soon as possible.

SPRAYING SURFACES WITH INSECTICIDE SOLUTIONS

Sets of tiles and egg-box collectors were sprayed with solutions of 5 % DDT, 5 % dieldrin and 5 % and 10 % BHC, sufficient to give a minimum dosage of 200 mg. of insecticide/ft.² (400 mg. in the case of 10 % BHC). Controls were sprayed with solvent only. After drying for 24 hr. the tiles and collectors were put out at L.W.E.S.T. on Bush Shore and Common Shore respectively (see map, Text-fig. 2). After 3 weeks the tiles were lifted. There was a heavy set of barnacles on both treated and control surfaces. The egg-box collectors were left for the whole of



Text-fig. 1. Concrete slab with protecting wire hoops, showing the arrangement of five quarry tiles.



Text-fig. 2. Map of Rivers Roach and Crouch at H.W.N.T. showing the oyster grounds (■) referred to in the text.

the oyster settling season but again there was no significant difference in the number of barnacles, or of oyster spat, settling on the treated and control surfaces. No counts were made of barnacles on the collectors as settlement had been interfered with by heavy silting in some of the interstices of the boxes. Instead, settlement was judged by eye and expressed as a percentage coverage of the total *available* surface.

IMMERSING SURFACES IN INSECTICIDE SOLUTIONS

The procedure was to immerse the tiles in the solutions for $\frac{1}{2}$ min. in which time a tile absorbed approximately 400 mg. of DDT. Tiles soaked for longer periods absorbed up to 1300 mg. of the insecticide.

On 11 July five blocks each bearing five tiles, which had been dipped in either the solutions or the solvent, were set out at L.W.E.S.T. on Purleigh Shore in the River Crouch. Frequent counts were made of the numbers of barnacles, spat and other sedentary organisms on each tile. Although the very intensive barnacle settlement had passed its peak, there was still a heavy fall which increased in the early part of October but had practically ceased by 27 October, when the tiles were finally lifted.

TABLE 1. *Numbers of barnacles on 15 cm.² of each of five tiles dipped in insecticide solutions*

Date	Treatment				
	5% DDT	5% BHC	10% BHC	5% dieldrin	Control
18. vii. 50	2	3	1	17	63
25. vii. 50	3	2	4	22	176
31. vii. 50	10	6	6	43	235
8. viii. 50	7	5	2	35	400
12. viii. 50	12	4	4	50	356
15. viii. 50	12	3	2	60	268
21. viii. 50	12	2	—	113	382
26. viii. 50	—	5	3	73	316
5. ix. 50	—	1	—	76	321
11. ix. 50	2	1	2	112	326
15. ix. 50	—	1	2	92	337
22. ix. 50	6	6	2	142	468
3. x. 50	2	2	1	119	523
10. x. 50	3	—	7	179	538
20. x. 50	—	—	8	185	575
27. x. 50	7	3	4	186	589

The numbers of barnacles on the control tiles rose rapidly in the first 3 weeks, and then after a slight fall remained almost constant until October, when they rose again (Table 1). Many barnacles died during the first few weeks after metamorphosis, but many survived throughout the experiment and grew to adult size. The mortality was probably somewhat greater than normal because of the proximity of the insecticide-treated tiles (see p. 413). On the dieldrin-treated tiles the barnacle population also increased, but the increase was not so rapid and mortality was greater. DDT and BHC were still more effective; although they did not prevent

barnacle settlement altogether they did cause 100 % mortality among the metamorphosed individuals with a few days. At the end of the experiment none of the tiles treated with DDT or BHC bore any barnacles greater than 1.5 mm. in rostral-carinal length and these were few and widely scattered. On the dieldrin-treated tiles the number of well-grown barnacles was considerable, while on the control tiles survival was better still (Pl. 16, fig. 3).

Other organisms settling on the tiles included *Crepidula fornicata* which appeared on them in considerable numbers from 25 July to 19 August and then disappeared. The size of some showed that they were newly settled while others were larger, crawling individuals. They did not exhibit any discrimination between differently treated surfaces. On 26 August, hydroid colonies appeared but also showed no discrimination; some of the colonies spread rapidly but the majority after spreading for a few weeks, died. The most flourishing colony appeared on F3 on 26 August and had covered the tile by 15 September after which it gradually died away; less than half the tile remained covered on 20 October and the colony had disappeared by 27 October. The only hydroid remaining on the tiles at the end of the experiment was *Tubularia larynx*. Colonies of *Flustrella hispida* (Bryozoa) appeared occasionally after 3 October, but only on the treated tiles. They suffered a slight mortality, but most survived and grew till the experiment was completed.

Ten oyster spat settled; all on treated tiles. They died and it is not possible to say whether death was natural or caused by the insecticides, although the results suggest that the insecticides were responsible. The tiles developed a film of some small algae, probably diatoms, which were grazed by *Littorina littorea*. The feeding tracks were easily seen but no dead individuals were observed on or around the tiles.

The placing of egg-box collectors on Purleigh Shore was delayed until some of the poles used for making the framework were varnished. The collectors were not put out until 11 August when the spatfall of oysters and settlement of barnacles had passed its peak. Like the tiles, the collectors were dipped, eight in each of the four solutions and eight in solvent, giving a total of forty. They were lifted and broken up on 14 November, the spat counted and the barnacles estimated as before (Table 2). Four collectors had broken away from the framework and were not recovered.

TABLE 2. *Percentage coverage by barnacles and, in brackets, number of spat on the dipped egg-boxes exposed on Purleigh Shore*

Solution								
5 % DDT	0 (2)	0 (1)	0 (3)	0 (3)	5 (8)	Lost	5 (4)	10 (0)
5 % BHC	5 (5)	5 (20)	65 (8)	10 (3)	Lost	5 (17)	0 (1)	0 (3)
10 % BHC	20 (0)	0 (59)	70 (7)	0 (25)	25 (1)	5 (3)	40 (0)	Lost
5 % dieldrin	55 (1)	15 (31)	30 (29)	25 (5)	75 (2)	5 (18)	40 (10)	50 (6)
Control	75 (54)	70 (50)	Lost	70 (7)	80 (16)	75 (3)	80 (0)	80 (21)

SURFACE COATINGS INCORPORATING INSECTICIDE

Three plain cardboard egg-box collectors were sent to British Industrial Plastics, where they were coated with varnish alone, varnish plus DDT and varnish plus BHC respectively, and then baked in a controlled temperature. All three were put out on the shore on 31 July. A month later it was easy to distinguish the boxes at a glance; the one treated with DDT bore only a few small barnacles, that treated with BHC bore a relatively dense population of well-grown individuals, while the control was smothered with barnacles. At the end of October they were examined and found to have resisted the prolonged immersion satisfactorily. Unfortunately they could not be examined for oyster spat as the beacon supporting them was broken during the first week-end in November and they were swept away.

The baking of varnished surfaces is not always practicable, so tests were made on lacquers which could be dried in air. To obtain results comparable with those of the previous experiment quarry tiles were painted with these compounds on 17 August, air dried and put out on Bush Shore. Of the five tiles on each block, two were varnished with lacquer containing DDT and two with BHC, while one was left untreated. One of each pair was placed with the smooth varnished side uppermost, the other, and the control, faced downwards. The tiles were exposed until 27 October and were examined at intervals. The records obtained were marred by the fact that the varnishes tended to blister and peel off. This was almost certainly because the surface was unsuitable for the varnishes, since no such tendency was observed when they were applied to wood. Settlement on the surfaces which faced upwards was masked by silt and weed so that it was not always possible to ascertain whether the barnacles were attached to the varnish or bare tile. It was easier to examine those which faced downwards and to make allowances where partial peeling had occurred. The results are given in Table 3, and show that while the varnish plus DDT caused 100 % mortality and virtually prevented barnacles settling, the varnish plus BHC reduced the settlement by approximately 75 % but did not cause such a great mortality.

TABLE 3. *Combined totals for settlement on 15 cm.² of each of five tiles set out on Bush Shore (only those tiles placed varnished surface downwards are considered)*

Date	DDT	BHC	Control
25. viii. 50	—	—	667
31. viii. 50	20	79	1842
11. ix. 50	6	543	1676
25. ix. 50	3	311	1768
3. x. 50	1	389	1683
10. x. 50	—	69	1601
20. x. 50	—	314	1383
27. x. 50	—	283	1375

Some of the poles forming the framework on Purleigh Shore were painted with one or other of these varnishes on 10 August. By mid-October it was easy to

distinguish those to which DDT had been applied because of the absence of barnacles. Those treated with BHC were almost as densely covered as the untreated ones. One pole was treated with two replicates of 1 ft. lengths in the order: unvarnished, varnish incorporating DDT, varnish incorporating BHC, then dried and fastened horizontally to the framework in such a position that it would not be chafed by the collectors. The pole, after 3 months' exposure to moderate barnacle settlement is shown in Pl. 16, fig. 2. It shows clearly that DDT is an efficient anti-fouling compound while BHC is of considerably less value. The photographs are of the underside of the pole, because the upper surface was masked by weed (*Enteromorpha* sp.), but there was considerably less weed on the parts with no barnacles. It is not known whether this was because the barnacles presented a better (rougher) surface for attachment or whether the DDT was directly harmful to the weed.

DISCUSSION

These experiments have shown that of the insecticides tested, DDT is the most suitable for preventing barnacle fouling while BHC, when used for dipping, can be almost equally efficient. Dieldrin does not seem to be of use as an anti-fouling agent. Loosanoff (1947) found that DDT did not greatly affect *Gryphaea virginica*, but *Ostrea edulis* appears to be much more sensitive to this insecticide. Further experiments will be made to determine whether DDT can be used selectively against *Elminius modestus* in the presence of oyster spat.

The results obtained from dipping and spraying the surfaces indicate that the quantity of active insecticide on each unit of surface area must be accurately determined. Spraying may have been ineffective because most of the insecticide was absorbed below the surface of the tiles and collectors. Dipping of surfaces may prove to be more successful and economical as the dosage per unit area can be more easily regulated.

As insecticide from the treated surfaces may occur in the water round the controls and affect settlement, the relative positions of control and treated tiles and collectors were varied. The settlement on the egg-box collectors was very variable, but results obtained with tiles suggest that sufficient insecticide to interfere with barnacle settlement, had dissolved into the water. In the experiment with the dipped tiles (Pl. 16, fig. 3) a control tile occupied the central position on block G while a dieldrin-treated tile was in the centre of block H. The number of barnacles settling on these centre tiles was less than the average for the similarly treated ones. This reduced settlement is well illustrated in Pl. 16, fig. 3, where the tiles occupy the positions G5 and H4 respectively. It was found that the number of barnacles on these two tiles never exceeded the average for the five. Since *E. modestus* prefers sheltered positions, its sparsity on the sheltered centre tiles suggests that the surrounding ones, treated with DDT and BHC, were releasing effective concentrations of insecticide into the water. Possibly the placing of treated surfaces around

untreated collectors might prove effective in reducing barnacle settlement while still permitting settlement of oysters.

The urea-formaldehyde resin combined with DDT prevents barnacle settlement over a period of at least 3 months and appears to withstand prolonged immersion extremely well. It will be useful in preventing barnacle fouling of the frameworks supporting collectors. This is important because of the gregarious tendency exhibited by *E. modestus* (Knight-Jones & Stephenson, 1950). Fewer barnacles settle on surfaces away from large populations of adult barnacles though any wooden structure left for some weeks in Essex creeks, even on muddy ground where there were no adult barnacles, would eventually, become smothered with *E. modestus*. Egg-box collectors suspended from such a structure would become covered by barnacles more rapidly than if fixed to supports that had been kept clean by varnishing with a lacquer containing DDT. Such a framework erected on a muddy shore where *E. modestus* is absent, but where large numbers of oyster larvae appear in the water, could be expected to collect more oyster spat.

The importance of *E. modestus* as the dominant fouling organism in Essex is well illustrated by the fact that it was the only barnacle found surviving on the tiles and poles used at Purleigh Shore. Some *Balanus improvisus* were seen on the collectors at Common Shore, but these were local in occurrence and few in number, compared with *Elminius modestus*. The latter is the chief obstacle to improvements in methods of collection of oyster spat, but results so far obtained indicate that there is a possibility of combating it successfully.

We thank Mr P. Bracey for his advice on the use and handling of these insecticides and for permission to use his formulae for the surface coatings. We are also indebted to Imperial Chemical Industries, to Julius Hyman and Co. and to British Industrial Plastics Ltd., for supplying insecticides and materials.

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Fig. 1.

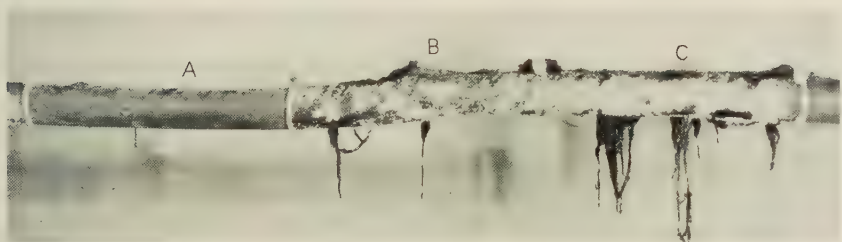


Fig. 2.

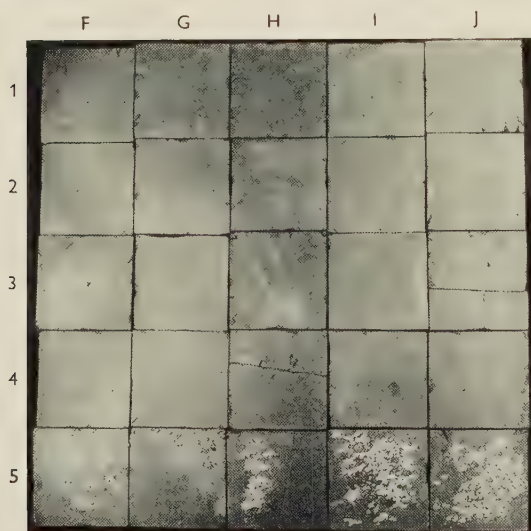


Fig. 3.

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EXPLANATION OF PLATE 16

- Fig. 1. Egg-box collectors on framework at Purleigh Shore, River Crouch.
- Fig. 2. Enlargement of centre section of pole, marked × on fig. 1; shows complete absence of barnacles on surface painted with varnish incorporating DDT. A, varnish + DDT; B, varnish + BHC; C, untreated control.
- Fig. 3. Dipped quarry tiles after 4 months' exposure at low-water mark on Purleigh Shore. Vertical rows: sets of tiles from blocks F to J. Horizontal rows: similarly treated tiles; 1, 5% DDT; 2, 5% BHC; 3, 10% BHC; 4, 5% dieldrin; 5, control.

(Received 23 January 1952)

PROCEEDINGS OF THE ASSOCIATION OF APPLIED BIOLOGISTS

Joint Meeting of the Association with the Crop Protection Panel of the Society of Chemical Industry held on Friday, 14 March 1952, in the Imperial College of Science and Technology, London; chairman at the morning session Mr W. E. K. Piercy, Chairman of the Crop Protection Panel, and at the afternoon session the President, Mr R. W. Marsh.

MORNING SESSION: *Herbicides*

1. The relative toxicity of nitrophenols to various organisms. By Dr E. W. SIMON.
2. The phytotoxicity of mineral oils and hydrocarbons. By Mr G. W. IVENS.
3. The effects of 'hormone' herbicides upon cereal crops. By Mr E. B. SCRAGG.

AFTERNOON SESSION: *Systemic fungicides*

4. Introduction. By Prof. R. L. WAIN.
5. Techniques in the bioassay of systemic fungicides. By Mr S. H. CROWDY.
6. Antibiotics as systemic fungicides and bactericides. By Dr P. W. BRIAN.
7. The evaluation of systemic fungicides by means of *Alternaria solani* on tomato. By Dr J. STUBBS.
8. Root injury as a factor in the assessment of chemotherapeutants by the tomato *Fusarium* wilt test. By Dr W. G. KEYWORTH.

HERBICIDES

THE RELATIVE TOXICITY OF NITROPHENOLS TO VARIOUS ORGANISMS

By E. W. SIMON, *King's College, London**

The effect of increasing degrees of nitration on the toxicity of phenols was assessed by measuring the toxicity of phenol, *o*- and *p*-nitro phenols and 2:4-dinitro phenol with several different test organisms. Toxicity levels were determined by the concentration required in each case to bring about the following responses: (i) to halve the rate of radial growth of *Trichoderma viride* at pH 3, (ii) to halve the respiration rate of yeast at pH 3, (iii) to halve the rate of frond multiplication of *Lemna minor* at pH 5.4, (iv) to bring about 50% mortality of seedlings of *Brassica alba* in a spraying test and finally (v) to halve the rate of respiration of leaf disks of *B. alba* vacuum-infiltrated with the toxic solution at pH 3.

The figures obtained in these various tests are not strictly comparable since some of them reflect differences in toxicity consequent on the varying degrees of ionization at the pH of the test as well as intrinsic differences in toxicity level (Simon & Blackman, 1949). Most of the

* This work was carried out while the author was a member of the Agricultural Research Council Unit of Experimental Agronomy, Oxford.

tests were carried out at pH 3, a pH at which none of the compounds is much dissociated, but at the pH of the *Lemna* test, 2:4-dinitro phenol (pH=4.0) was present largely in the dissociated form and in consequence a relatively high concentration of 2:4-dinitro phenol was required in this test. Since it was not possible to make a direct determination of the toxicity of 2:4-dinitro phenol to *Lemna* at pH 3, it was necessary to derive an estimate from the experimentally determined figure at pH 5.4 by means of the general relationship between pH and toxicity given by Simon & Beevers (1952). A comparable problem arose in dealing with the results of the spraying experiments. Here the pH at which the spray is applied matters less than the pH at the site of action of the toxic substances within the cells, since the tissue as a whole has a greater buffer capacity than the spray droplets (Simon, Roberts & Blackman, 1952). It was assumed that the appropriate pH would be approximately 6 and a figure for the toxicity of 2:4-dinitro phenol at pH 3 was estimated on this basis.

Toxicity comparisons at pH levels at which there was little dissociation showed that relative toxicity was in large measure independent of the test organism. Phenol was the least toxic compound in each test, *p*-nitro phenol being about 40 times more toxic, and 2:4-dinitro phenol 3200 times more toxic. In a test with yeast, 2:4:6-trinitro phenol proved to be less toxic than dinitro phenol. *o*-Nitro phenol was consistently less toxic than the *p*-isomer, and in the spraying experiment no toxicity at all was recorded with the *o* compound. This toxicity difference is probably related to the fact that *o*-nitro phenol is more volatile and less soluble than the *p*-isomer.

Measurements were made with the *Trichoderma* test of the toxicity of four other nitration series based respectively on anisole, phenetole, toluene and nitrobenzene. There was a fair degree of similarity in the effect of increasing nitration in these series, the change from parent compound to mononitro and from dinitro to trinitro derivative being accompanied by large increases in toxicity, whilst the dinitro compound was not much more toxic than the mononitro.

Further analysis of the results was made along the lines suggested by Ferguson (1939) and Albert (1951) by comparing the concentrations required to produce the toxic effect with such solubility values for the compounds as are available in the literature. The nitro anisole series, the nitro toluene series, picric acid and nitro benzene were only toxic in solutions approaching the saturated, having values of activity (solubility/toxicity) above 0.1. Such substances are believed to have only a 'physical' action acting as foreign bodies within the cell. On the other hand, phenol, the mono- and di-nitro phenols and the di- and tri-nitro-benzenes were toxic in much weaker solutions, having activity values between 0.002 and 0.06: such compounds are structurally specific, their high degree of toxicity depending on a quite specific chemical structure.

The work of Loomis & Lipmann (1948) and of Cross, Taggart, Covo & Green (1949) suggests that the chemical specificity of the active nitro compounds is related to an uncoupling of oxidative phosphorylation. In agreement with the requirements of this hypothesis, it was found that higher concentrations of each compound were needed in the tests based on 50% reduction of respiration rate than in those concerned with 50% reduction in the growth rate. Since growth is dependent on an energy supply, concentrations of nitro compound which dislocate oxidative phosphorylation would affect growth, although the respiration rate is increased by such concentrations: only with higher concentrations is there a reduction of the respiration rate.

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THE PHYTOTOXICITY OF MINERAL OILS AND HYDROCARBONS

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Mineral oils are being increasingly used as herbicides, and there is little doubt that they could be used more effectively if the phytotoxic properties of any particular fraction could be predicted from knowledge of its physical and chemical properties without the need for extensive testing in the field. This paper reviews some of the work that has been done on the correlation of toxicity with physical and chemical properties of oils, and presents some of the results obtained in an investigation into the toxicity of mineral oils and hydrocarbons to plants, being carried out by the Agricultural Research Council Unit of Experimental Agronomy.

Some of the earliest work on the toxicity of mineral oils to plants was done in connexion with their use as emulsions to kill insect pests on fruit trees (Martin, 1935). It was found that there was some correlation between the degree of injury caused by the emulsions to the trees and the unsulphonated residue of the oil. Oils with a high unsulphonated residue (i.e. those consisting largely of paraffin and cycloparaffin hydrocarbons) were generally safer than those with a relatively high proportion of aromatic compounds. There was also some relation between toxicity and viscosity. With the light lubricating oils generally used for fruit-tree spraying, there was a tendency for toxicity to increase with viscosity up to a point and then to fall. The unsulphonated residue and viscosity therefore gave some guide to the selection of oils for use as sprays, but it was evident that these characters alone were not sufficient as oils of high unsulphonated residue sometimes caused considerable injury to the trees.

Oils used as selective weed killers are applied in the pure, unemulsified form, but a similar rough correlation between toxicity and aromatic content has been found to exist. Highly refined oils of low aromatic content are generally of low toxicity. Oils of moderate aromatic content (10–30%) are of moderate toxicity and include most of the vaporizing oils and white spirits used as selective herbicides. Highly aromatic oils, such as the aromatic extracts consisting of aromatic compounds removed during the refining of other fractions, are generally highly toxic. Toxicity is also correlated to some extent with the boiling range of the oil. Heavier oils, such as diesel and gas oil, are in general more toxic than vaporizing oils (boiling from about 180 to 250° C.) of similar aromatic content, and these in turn are somewhat more toxic than white spirits (boiling range 150–200° C.).

If the toxicities of oil fractions, prepared either by the fractionation of a single oil or by refining an oil to different degrees, are compared, these relationships hold quite well. If oils from different sources are compared, however, the correlations are often much less close, and fractions of very similar boiling range and aromatic content may differ considerably in their toxicity to plants. Thus, although the general types of oil which are most suitable for various herbicidal purposes are known, knowledge of boiling range and aromatic content is not enough to enable the suitability of particular samples to be determined.

Mineral oils consist of mixtures of large numbers of hydrocarbons. Their precise composition and physical properties depend largely on the type of crude from which they have been obtained and on the details of the refining processes to which they have been subjected. As both of these factors are liable to change, even successive batches of one particular oil fraction may vary to some extent in their phytotoxic properties. For this reason a number of workers have studied the relationships between phytotoxicity and properties of the individual hydrocarbons of which oils are composed.

Comparisons of the toxicity of hydrocarbons can be made in various ways, and the results obtained depend to some extent on the method by which the compounds are applied. In most of the work so far reported, hydrocarbons have been sprayed, either in the pure form or diluted in some way, on to plants and the toxicity assessed either by the percentage of plants killed or by estimates of the degree of damage.

When liquid hydrocarbons are applied by spraying in the undiluted state the amount of damage caused and the estimates of toxicity obtained depend to some extent on factors other than their intrinsic toxicity, such as the degree of penetration and spreading within the leaves of the plant. The amount of liquid which penetrates into the leaves, for instance, is determined partly by the volatility of the compound. With the more volatile liquids, such as benzene, much less of the liquid enters into the leaves than with compounds of higher boiling-point and their toxicity may be underestimated. Similarly, penetration into the leaves and spreading through the intercellular spaces are dependent on such factors as the viscosity, surface-tension, and contact angle of the liquids so that differences in these properties will also affect the estimates of relative toxicity.

When toxicities are compared by measuring the concentrations of various hydrocarbons in some non-toxic diluent that are required to cause a certain degree of injury the properties of the diluent must also be taken into account. Water has been used as a diluent, by applying the hydrocarbons as emulsions, but this is not very satisfactory as oils, when emulsified, are known to have different phytotoxic properties to unemulsified oils. On the other hand, none of the diluents so far tested which are miscible with hydrocarbons are entirely non-toxic. The least toxic are themselves hydrocarbons, either highly refined paraffinic oils or relatively heavy paraffins such as *n*-tetradecane. By using diluents of this type, differences in the properties of hydrocarbons which influence their penetration and spread are reduced and the estimates of toxicity obtained are rather different to those obtained with the undiluted compounds. For instance, some highly toxic compounds, such as the higher boiling-point aromatics, do not appear to spread extensively over plant surfaces in the pure state. When diluted, however, they spread over a wider area and cause more damage. In experiments with diluents, therefore, these compounds may appear to have a higher degree of toxicity than when they are applied pure. With highly volatile compounds, also, there is a tendency for experiments with diluents to give higher estimates of toxicity.

Extensive comparisons of the toxicity of hydrocarbons applied as sprays have been made by Havis (1948, 1950) and Leonard & Harris (1950). Havis used both the undiluted compounds and dilutions in a highly refined oil of low toxicity. Applying the compounds in either form, there was an increase in toxicity with boiling-point up to a maximum, followed, particularly in the paraffin series, by a decrease in toxicity with higher members. In general, the aromatic series appeared to be most toxic, the olefines and cycloparaffins intermediate, and the paraffins least toxic. Although the estimates of toxicity obtained with the pure and diluted hydrocarbons showed similar trends, there were some considerable differences. For example, the olefine *n*-decene-1, when applied pure appeared to be of about the same toxicity as the aromatics diethylbenzene or triethylbenzene. When diluted however, *n*-decene-1 was much less toxic than the benzene derivatives, causing no injury at 30–35 %, whereas plants could only tolerate 5–10 % of diethylbenzene or 2–3 % of triethylbenzene.

Leonard & Harris, in experiments with benzene derivatives diluted with a highly refined oil, also demonstrated that the toxicity increased with addition of methyl groupings up to pentamethylbenzene. With paraffins and olefines applied in the undiluted form, toxicity

increased with boiling-point up to compounds with twelve carbon atoms and then decreased. Olefines were more toxic than paraffins of similar boiling-point.

These experiments suggest that, at least with the lower boiling-point compounds, there is some relation between boiling-point and toxicity. With experiments of this type, however, in which hydrocarbons are applied in the liquid form, it is difficult to judge to what extent the relationship is based on differences in the toxicity at the site of action and how far it is determined by differences in such factors as volatility, surface-tension and viscosity which determine the degree of penetration and spreading and are themselves related to boiling-point. The experiments also show that the chemical nature of hydrocarbons influences their toxicity. Differences in chemical structure, however, are also reflected in such properties as surface-tension and contact-angle which affect the access of the compounds to the site of action. It is therefore again difficult to determine how closely the differences between the various groups of hydrocarbons are related to differences in toxicity at the site of action.

In a series of experiments at Oxford an attempt has been made to eliminate the factors influencing the access of hydrocarbons to the cells by applying the compounds in the vapour phase. The method used was to expose detached leaves of *Phaseolus multiflorus* (runner bean) to a series of concentrations of different vapours, obtained by volatilizing a measured volume of liquid into a bell-jar containing the leaf. Preliminary experiments showed, that over a range of treatment times from 15 min. to 2 hr. the time of exposure had little effect on the response of the leaves. The leaves were therefore exposed to all the treatments for a standard time of 1 hr. On treating leaves with a range of concentrations of a hydrocarbon by this method it was found that there was a relatively small difference between concentrations causing no damage and those completely killing the leaves. It was therefore possible to obtain relatively precise and reproducible estimates of the concentrations causing 50 % injury to the leaves and the compounds were compared on the basis of these LD 50 estimates. The results obtained with the aromatic series of compounds are presented in Table 1.

TABLE 1. *The toxicity of various aromatic hydrocarbons applied as vapours to leaves of runner bean and parsnip at 25° C.*

Compound	Boiling-point (° C.)	Runner bean				Parsnip		
		Saturated vapour pressure	Conc. at LD 50 (mols./l. $\times 10^5$)	Vapour pressure at LD 50 (mm. Hg)		Conc. at LD 50 (mols./l. $\times 10^5$)	Vapour pressure at LD 50 (mm. Hg)	
		(mm. Hg) p_s		Degree of saturation p_t/p_s			Degree of saturation p_t/p_s	
Benzene	80	95.2	154	28.6	0.30	269	50.0	0.53
Toluene	111	28.5	47.5	8.83	0.31	96.7	18.0	0.63
Ethyl benzene	136	9.52	25.5	4.73	0.50	45.2	8.40	0.88
<i>p</i> -Xylene	138	8.76	22.9	4.26	0.49	37.7	7.01	0.80
<i>o</i> -Xylene	144	6.62	18.4	3.42	0.52	27.6	5.13	0.78
Isopropyl benzene	152	4.57	17.1	3.17	0.69	*	*	> 1.0
Mesitylene	165	2.42	12.7	2.37	0.98	*	*	> 1.0
Pseudocumene	169	2.03	10.4	1.94	0.96	—	—	—
<i>m</i> -Diethylbenzene	181	1.08	*	*	> 1.0	—	—	—
Diisopropylbenzenes	205	0.3	*	*	> 1.0	—	—	—

* Not toxic at saturation.

It is evident from these results that the toxicity of hydrocarbon vapours to bean leaves increases with the boiling-point of the compound. If the logarithm of the LD 50

concentration is plotted against the boiling-point, there is a close approximation to a straight-line relationship and, since the saturated vapour-pressure is closely related to boiling-point, there is also an inverse relationship between the logarithm of the LD 50 concentration and the logarithm of the vapour-pressure. These relationships, however, only hold for the lower members of the series, as compounds with boiling-points above about 170° C. (or with vapour pressures below about 2 mm. of mercury at 25° C.), either caused only slight damage or were not toxic in the vapour phase. *m*-Diethylbenzene, for instance, with a boiling-point of 181° C. caused very little injury even as a saturated vapour.

This sharp 'cut-off' in the increase of toxicity with boiling-point is explained by considering the relation between the vapour-pressure at the LD 50 concentration and the saturated vapour-pressure, i.e. the degree of saturation of the air with hydrocarbon vapour. It is evident from the table that, with increasing boiling-point, this ratio also rises until, at about 170° C. the value reaches unity. At this point a completely saturated vapour is required to cause the effect being measured and compounds of higher boiling-point do not produce the effect at all. Table 1 also contains the results of a similar series of experiments with parsnip leaves. This plant is relatively resistant to oil sprays in the field and it is of interest to note that higher concentrations of hydrocarbon vapours are required to cause a comparable degree of injury than with bean leaves. Similar relationships between toxicity and boiling-point or vapour-pressure exist and, as higher concentrations are needed to cause injury, it follows that the 'cut-off' occurs with compounds of lower boiling-point. With parsnip leaves, *isopropylbenzene* and compounds of higher boiling-point were not toxic as saturated vapours.

Tables 2 and 3 show the results obtained with other types of hydrocarbon and, although the relation between saturated vapour-pressure and the degree of saturation of equitoxic concentrations is not so clearly demonstrated as with the aromatic series, in general the same relationships can be observed. With the paraffins (Table 2) it is seen that *n*-heptane with a boiling-point of 98° C. is more toxic than *iso*-octane, which has a slightly higher boiling-point. *Iso*-octane, however, has a higher vapour-pressure than *n*-heptane so that it is probable that the toxicity is determined by the vapour-pressure rather than by the boiling-point.

TABLE 2. *The toxicity of various paraffin and cycloparaffin hydrocarbons applied as vapours to leaves of runner bean and parsnip at 25° C.*

Compound	Boiling-point (° C.)	Saturated vapour pressure (mm. Hg) p_s	Runner bean			Parsnip		
			Conc. at LD 50 (mols./l. $\times 10^5$)	Vapour pressure at LD 50 (mm. Hg) p_t	Degree of saturation p_t/p_s	Conc. at LD 50 (mols./l. $\times 10^5$)	Vapour pressure at LD 50 (mm. Hg) p_t	Degree of saturation p_t/p_s
Cyclohexane	81	97.6	297	55.2	0.57	—	—	—
<i>Iso</i> -octane	99	49.3	140	26.1	0.53	—	—	—
Methyl- cyclo- hexane	101	46.3	121	22.6	0.49	192	35.6	0.77
<i>n</i> -Heptane	98	45.7	93.9	17.5	0.38	230	42.7	0.93
<i>n</i> -Octane	126	14.0	35.0	6.51	0.46	*	*	> 1.0
Ethylcyclo- hexane	131	12.8	40.8	7.58	0.59	66.4	12.4	0.97
2:7-Di- methyl- octane	160	3.6	17.6	3.27	0.91	*	*	> 1.0
<i>n</i> -Decane	174	1.3	*	*	> 1.0	—	—	—

* Not toxic at saturation.

TABLE 3. *The toxicity of various olefine hydrocarbons applied as vapours to leaves of runner bean at 25° C.*

Compound	Boiling-point (° C.)	Saturated vapour pressure (mm. Hg) p_s	Conc. at LD 50 (mols./l. $\times 10^5$)	Vapour pressure at LD 50 (mm. Hg) p_i	Degree of saturation p_i/p_s
Cyclohexene	83	90	147	27.4	0.30
Diisobutylene	102	40	106	19.7	0.49
<i>n</i> -Octene-1	121	17.5	49.1	9.12	0.52
<i>n</i> -Octene-2	125	15.6	46.8	8.70	0.56
Vinylcyclohexene	129	13.3	34.3	6.37	0.48
<i>n</i> -Decene-1	172	2.4	10.0	1.86	0.78
Triisobutylene	178	1.4	*	*	> 1.0

* Not toxic at saturation.

The values of the degree of saturation at equitoxic concentrations are related to the thermodynamic activities. As pointed out by Ferguson (1939), the thermodynamic activity is a useful measure of toxicity as, at equilibrium, it is unaffected by partition differences between different phases and the activity at the site of action is the same as that in the phase in which the toxic substance is applied. The activity also gives some indication of the type of action of toxic compounds. For substances which enter into chemical reactions and directly interfere with metabolic processes, the activity values are generally considerably less than 0.1. On the other hand, substances whose toxicity depends on certain physical properties which lead to their accumulation in some phase of the organism and which thereby interfere with some vital process without taking part in any chemical reaction, generally have activity values between 0.1 and 1.0. This latter type of substance has been referred to as structurally non-specific (Albert, 1951). With all the hydrocarbons tested in these experiments the values lie between 0.3 and 1.0, and there are good grounds for classing hydrocarbons as structurally non-specific substances which do not depend on any specific chemical grouping for their activity. The abrupt 'cut-off' in toxicity noted in these experiments is also a frequent characteristic of homologous series of this class of compound.

Comparing the different types of hydrocarbon it is evident that, in the vapour phase, there are no very large differences in toxicity. There are individual differences between compounds of very similar vapour-pressure; cyclohexane, for instance, appears to be considerably less toxic than either benzene or cyclohexene, but in general it is not possible to say that any one class of compound is more toxic than another.

This finding is in marked contrast to the results obtained from experiments using hydrocarbons or oils as liquids, where there is considerable evidence of differences in the toxicity of different groups of compounds. It is also apparent that many hydrocarbons of higher boiling-point which are not toxic in the vapour phase, are extremely toxic as liquids. The toxicity of vapours must therefore depend to some extent on different factors to that of liquids but, until more evidence is available on the site and mode of action of these compounds, it will be difficult to correlate the results obtained by applying hydrocarbons in the two forms.

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THE EFFECTS OF 'HORMONE' HERBICIDES UPON CEREAL CROPS

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INTRODUCTION

The most obvious phytocidal effect of 'hormone' herbicides upon cereal crops is in the production of morphological abnormalities in the vegetative parts and in the inflorescence. These are striking, but not necessarily so detrimental as possible effects upon yield and quality of grain.

The initial abnormality of the vegetative parts is a slight twisting and scorching of the leaf blade within a few hours of spraying. This rapidly disappears and the plant resumes normal growth. When shooting of the heads takes place, later in the season, a proportion may have difficulty in emerging from the boot, owing to the fusion of the leaf sheath of the uppermost leaf. This gives characteristically 'bowed' ears, especially in barley. 'Bowed' ears finally emerge from the boot and are usually quite normal.

Damage to the ears of wheat and barley frequently takes the form of pairing of the spikelets, instead of the normal alternate arrangement. Supernumerary smaller spikelets may be formed below the normal ones or a whorl of three or more spikelets may be present at one node.

In wheat, fusion of the outer edges of the glumes produces an effect which has been called 'big glume'. A somewhat similar effect results in barley, when the normally sterile lateral florets, of a two-rowed ear, are rendered fertile. Their pales fuse with those of the central spikelet, giving a single large inflated outer pale, which may carry a triple awn. This damage has been called 'tweaking' of the ears; it results in the formation of a compound grain containing three embryos. Such large grains, by causing irregularity of the sample, may lower the quality of grain produced.

Morphologically abnormal ears are less common in oats and the damage found is much less severe. The most frequent abnormality is the presence of three empty glumes instead of the normal two. After late spraying of oats, the panicles may show an appearance that has been called 'featheriness'. The branches of the inflorescence are erect and spreading instead of drooping; the glumes are white and papery, with a green base instead of the normal uniform golden yellow colour. This type of abnormality is associated with considerable sterility of the florets.

FACTORS AFFECTING THE PRODUCTION OF MORPHOLOGICAL ABNORMALITIES

Time of application of herbicides in relation to stage of crop development

The nature of the damage at once suggests that 'hormones' interfere with the normal processes of differentiation of primordia from the growing point. The time of maximum susceptibility is at the stage of development during which the spikelet primordia are formed.

Table 1 shows the results of applying Agroxone (single strength)—(contains sodium salt of 2-methyl-4-chlorophenoxyacetic acid MCPA or methoxone) at 1 gal./acre to *Kenia* barley, at eleven stages of growth. Maximum susceptibility to damage occurred when the plants were 5 in. tall, with three fully expanded leaves. This is the stage of development immediately before tillering. Damage occurred in the earliest-sprayed plots, and the period of susceptibility extended from soon after emergence until tillering had begun, approximately 3 weeks.

Examination of growing points showed that the stage of maximum susceptibility coincided with the change from the differentiation of leaf to spikelet primordia. Plants sprayed at this stage would be subjected to the action of the 'hormone' herbicide during the whole period of differentiation of spikelet primordia. By the end of the susceptible phase, the growing points showed complete differentiation of the spikelet primordia.

In this particular experiment, bowed ears occurred exclusively after the first three sprayings, i.e. during differentiation of the primordia of the upper leaves. By the time of the fourth spraying differentiation of leaf primordia had ceased and so bowed ears were not formed.

TABLE 1. *Results of Exp. E 1. Goughburn Field, 1950*

(Variety: Abed Kenia; date of sowing: 4 May 1950; herbicide: Agroxone (single strength), 1 gal./acre in 100 gal. spray.)

Spray no.	Days from sowing to spraying	Length of growing point (av. 5 plants) (mm.)	Stage of crop development	Damaged ears (%)
1	17	0.33	2-3 in. tall; 2 leaves visible	10.1
2	20	0.38	No visible increase in size over plot 1	13.8
3	23	0.44	3 in. tall; 3rd leaf emerging	17.7
4	26	0.70	5 in. tall; 3 fully expanded leaves	32.4
5	30	0.89	8 in. tall; beginning to tiller, rapid growth since 4th spray	19.7
6	34	1.37	10 in. tall; 4 fully developed leaves, 5th leaf visible	6.3
7	36	1.84	Little increase in size over plot 6	—
8	40	3.15	10-12 in. tall; 5 full leaves	—
9	44	3.43	12 in. tall; up to 7 full leaves, lower internodes elongating	—
10	47	*	12-15 in. tall; 7 full leaves, lower internodes 2-3 in. long	—
11	56	*	18 in. tall; shooting rapidly	—
Control, not sprayed	—	—	—	—

* Growing points too long to be measured by same technique.

TABLE 2. *Results of Exp. S 1 (wheat). Field 35, 1951*

(Variety: Fylgia; date of sowing: 11 April 1951; herbicide: methoxone, 1 lb. acid equiv./acre in 100 gal. spray.)

Spray no.	Days from sowing to spraying	Length of growing point (av. 5 plants) (mm.)	Stage of crop development	Damaged ears (%)
1	42	0.34	2 full leaves, 3rd leaf emerging	18.75
2	58	1.11	3 or 4 full leaves, 5th leaf emerging in some plants	4.0
3	82	4.50	6 full leaves, 7th leaf emerging; 12-18 in. tall	2.2
Control, not sprayed	—	—	—	3.5

In wheat (Table 2) the stage of maximum susceptibility to damage is even earlier than in barley. In the 'first spray' treatment, differentiation of the spikelet primordia had not begun, in the 'second spray' treatment it was well advanced and in the 'third spray' treatment completed.

The susceptible period in oats appears to be longer than in barley and wheat, and to occur at a later stage. In this crop differentiation of the spikelets extends over a longer period, and proceeds irregularly within the panicle. Thus the uppermost spikelets are completely formed

before differentiation of the lower ones has begun. Table 3 shows that a crop of *Star* oats first became susceptible to damage just before tillering, and continued susceptible until well after tillering, when it was 12-15 in. high, and had almost five full leaves.

TABLE 3. *Results of Exp. S 1 (oats). Field C 4, 1951*

(Variety: *Star*; date of sowing: 11 April 1951; herbicide: sodium salt of 2:4-D, 1 lb. acid equiv./acre in 100 gal. spray.)

Spray no.	Days from sowing to spraying	Stage of crop development	Damaged spikelets (%)
1	31	1-1½ in. tall; 1st true leaf emerging from coleoptile	0
2	35	2 in. tall; 1st true leaf not completely expanded	0
3	41	3-4 in. tall; 2 full leaves, 3rd leaf emerging	1.2
4	46	4-5 in. tall; 2 full leaves, 3rd leaf emerging. Early tillering stage	2.5
5	51	5 in. tall; 2 full leaves, 3rd leaf emerging, 4th leaf visible. Tillering	2.6
6	56	6-8 in. tall; 3 full leaves, 4th leaf emerging, 5th leaf visible	4.3
7	61	12-15 in. tall; 4 full leaves, 5th leaf emerging, 6th leaf visible	1.4
8	71	18-24 in. tall; 5 full leaves, 6th leaf emerging	0
Control, not sprayed	—	—	0

The effects of different herbicides and relative susceptibility of varieties

The herbicides at present in commercial use are based on methoxone, usually the sodium salt, and 2:4-D (2:4-dichlorophenoxyacetic acid). The latter is available in three forms, the sodium salt, an amine salt and an ester, usually the *n*-butyl ester. If these substances are used at normal rates of application, away from the susceptible period, they have no phytotoxic effect on cereals, but when applied at the stage of maximum susceptibility all cause damage.

Table 4 shows how different varieties reacted to methoxone and the three forms of 2:4-D, applied at 1 lb. acid equivalent per acre. The amine form of 2:4-D caused most, and the sodium salt of 2:4-D least, damage. The ester of 2:4-D caused less damage than the amine, and was very slightly more damaging than methoxone.

In general, 2:4-D is considered a more potent herbicide than methoxone. There are, however, exceptions to this rule. In an experiment, hemp nettle (*Galeopsis tetrahit*), an important weed in the north of Scotland, was killed by methoxone, but only slightly affected by all forms of 2:4-D.

THE EFFECT OF 'HORMONE' HERBICIDES UPON YIELD IN CEREALS

Templeman & Halliday (1950) report no significant reductions in yield in any cereal, with methoxone and sodium 2:4-D used at normal rates. At much higher rates, up to 16 lb./acre, the most susceptible periods were very early, and very late in crop development. In a later publication, Templeman & Wright (1951) show that both early and late applications of the *n*-butyl esters of methoxone and 2:4-D caused significant decreases in yield in oats and barley. The 2:4-D ester caused a greater reduction than the ester of methoxone, and late application was more dangerous than early.

Table 5 reports an experiment carried out at the College Farm using various proprietary selective herbicides at the recommended rates. These results show a significant decrease in yield, when the amine and ester of 2:4-D were used on oats at a late stage of growth. The decrease in yield was not associated with the presence of morphologically abnormal spikelets, but with sterility in the florets.

TABLE 4. *Results of Exp. S 2. Goughburn Field, 1951*

Wheat						
Treatment (1 lb. acid equiv./acre)	Percentage abnormal ears					
	Fylgia	Atle			Fylgia (S 1)	
Methoxone	8.75	3.75			18.75	
Sodium 2:4-D	2.0	1.75			—	
Amine 2:4-D	20.8	11.75			—	
Ester 2:4-D	11.5	9.75			40.5	
Control	1.5	0			3.5	

Barley						
Treatment (1 lb. acid equiv./acre)	Percentage abnormal ears					
	Earl	Plumage Archer	Spratt Archer	Kenia	Freja	Freja (S 1)
Methoxone	4.0	17.75	12.5	9.25	11.25	7.5
Sodium 2:4-D	0.25	6.25	2.25	1.75	1.0	—
Amine 2:4-D	17.5	26.25	21.25	31.0	22.75	52.7
Ester 2:4-D	5.75	26.25	21.25	9.5	12.75	—
Control	0	2.75	0	0.5	0	0

Oats						
Treatment (1 lb. acid equiv./acre)	Percentage abnormal ears					
	Onward	Victory	Supreme	Star	Star (S 1)	
Methoxone	0	0	1.3	0	0.6	
Sodium 2:4-D	0	0	0	0	—	
Amine 2:4-D	10.3	3.0	3.0	4.0	4.3	
Ester 2:4-D	0.6	0.6	1.3	2.0	—	
Control	0	0	0	0	0	

TABLE 5. *Results of Exp. on Field 47 (oats), 1950*

(Variety: Victory; date of sowing: 8 April 1950; date of spraying: 16 June 1950; stage of development: 16-18 in. tall. All herbicides applied by 'Agro' sprayer at 10 gal./acre.)

Treatment	Yield (qtr./acre)	Sterile spikelets (%)	1000-grain weight (g.)	Crude protein in dry matter (%)
Control. Not sprayed	11.27	2.1	37.8	14.8
Agroxone, 1 gal./acre (methoxone)	10.50	0.8	38.3	15.0
Sodium 2:4-D, $\frac{3}{4}$ lb./acre	10.42	3.4	40.2	15.1
Phenoxylenec, $\frac{1}{2}$ gal./acre (methoxone)	9.97	3.4	40.1	14.7
Sodium 2:4-D, 1 lb./acre	8.70	9.6	41.8	15.0
Cornox, 1 pt./acre (amine 2:4-D)*	7.09	59.2	45.4	17.2
Cornox, 2 pt./acre (amine 2:4-D)	4.40	64.4	44.7	18.5
Dicotox A, $\frac{1}{2}$ gal./acre (ester 2:4-D)†	3.60	90.2	47.0	19.0

* Average 2 plots only.

† N.B. Recommended by the manufacturers for wheat and barley only.

Moore (1950) reports decreases in yield of wheat of up to 10 bushels/acre following the application of the *n*-butyl ester of 2:4-D, at 1 lb./acre in 100 gal. spray, to a crop in the seedling (3-leaf) stage. He found that all phenoxyacetic acid compounds reduced yield, when applied at the seedling stage, the ester and amine of 2:4-D being most toxic. The reductions in yield were due to a decrease in number of ears. The ester of 2:4-D reduced yield of wheat and barley, when applied at the flowering stage by diminishing grains per ear rather than ears per plant. Late applications of sodium 2:4-D and methoxone, caused no reduction in grain yield, even at 5 lb./acre.

Anderson & Hermansen (1950) suggest that oats show the greatest decrease in yield and wheat the smallest, and that the sodium salt of 2:4-D causes a greater decrease than methoxone. They find that the dates for the greatest number of abnormalities and for the lowest yield coincide, but that sometimes a considerable proportion of abnormalities are found without corresponding decrease in yield.

This observation is confirmed by Table 6, which shows that even when the percentage of abnormal ears is very high, the yield may not be reduced. Since abnormality in barley is due to increased fertility of the spikelets, this result might be expected.

THE EFFECT OF 'HORMONE' HERBICIDES UPON QUALITY OF GRAIN

Erikson, Seely & Klages (1948) showed that the protein content of wheat was increased by hormone treatment, but Schellenberger, Phillips, Johnson & Miller (1950) recorded no significant difference in protein or mineral content, milling or baking quality following the application of 2:4-D.

Table 5 shows that, in an experiment where significant decreases in yield of oats were found, following treatment with various herbicides, 1000-grain weight and protein content increased, as yield decreased. Barley showed no increase in protein content (Table 6) even in the most severely damaged plots, but 1000-grain weight is increased by the 'first' and 'second spray' treatments with amine 2:4-D. Other workers have found a decrease, rather than increase, in 1000-grain weight, after spraying.

TABLE 6. *Results of Exp. S 1 (barley). Field 73, 1951*

(Variety: Freja; date of sowing: 19 April 1951.)

Spray no.	Days from sowing to spraying	Length of growing point (av. 5 plants) (mm.)	Stage of crop development	Damaged ears (%)		Yield (cwt./acre)		1000-grain weight (g.)		Crude protein in dry matter (%)	
				M.	D.	M.	D.	M.	D.	M.	D.
1	33	0.55	2-3 in. tall; 1 fully expanded leaf and 2nd nearly completely emerged	7.5	52.7	24.4	23.6	42.5	46.1	9.41	9.68
2	46	1.74	6-8 in. tall; 3 fully expanded leaves, 4th leaf emerging	1.0	1.0	27.7	30.4	42.5	45.5	9.23	8.96
3	71	22.0	18-24 in. tall; 7 full leaves, ear in boot	0	0	26.7	23.3	42.9	43.8	9.11	9.8
Control, not sprayed	—	—	—	0	0	24.0	22.0	42.7	43.3	8.38	9.33

M.=methoxone (sodium salt), 1 lb. acid equiv./acre. D.=2:4-D (amine salt), 1 lb. acid equiv./acre.

'Hormone' treatment does not, apparently, impair viability of the grain. In barley, the three embryos found in the large fused kernels are usually all capable of germination. A report from Rossman & Sprague (1949), however, states that vigour of the plants growing from seed produced by damaged ears of maize is reduced and yield lower than normal.

OTHER EFFECTS OF 'HORMONE' HERBICIDES

Other adverse effects of 'hormone' spraying which have been observed are uneven ripening, weakness of the straw and, in oats, secondary growth after harvest.

Soil and weather may influence herbicide damage. Certain soils retain 'hormone' herbicides longer than others, and dichloro compounds are broken down by soil micro-organisms more rapidly than trichloro or methyl compounds. Weather affects rate of growth, and it is recognized that plants in active growth are much more susceptible to damage than those which are retarded.

CONCLUSIONS

Spraying before tillering or immediately prior to emergence of the ear, should be avoided, so as to reduce liability to decrease in yield and abnormal ears. Unless the nature of the weed infestation demands it, rates of application greater than 1 lb. acid equivalent per acre should not be used; methoxone is less dangerous than the amine or ester forms of 2:4-D. Sodium 2:4-D appears to be relatively harmless but, owing to its low solubility, is not suitable for low volume spraying.

Finally, although 'hormone' herbicides may sometimes cause rather serious damage to cereal crops, in general the good resulting from the elimination of weeds far outweighs any harm. It should be remembered that the alternatives available: DNOC, copper chloride and sulphuric acid, are also liable to cause damage to cereals, and are more costly and difficult to apply.

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SYSTEMIC FUNGICIDES

INTRODUCTION

By R. L. WAIN, *Wye College, University of London*

The idea of controlling plant diseases by introducing curative chemicals into the plant goes back many years. All the early work, which has been reviewed by Müller (1926), was of an empirical nature and although claims were made from time to time that particular treatments were effective, no clear and reliable method ever became established for controlling a plant disease by internal therapy. Within the last few decades, however, such treatment for certain animal diseases with substances possessing antibacterial properties has given spectacular results. This success in the animal led to a renewed interest in the control of plant diseases by similar means, though it was of course recognized that the animal, with its circulating blood system capable of carrying chemicals rapidly to all tissues, is very different from the plant.

Not only has attention been given to the control of specific diseases in plants by chemicals acting systemically, but the control of insect pests and mineral deficiency conditions by such means has also been intensively investigated. In such studies, investigations on the distribution of compounds within the plant following application to roots, stems or leaves has been of great value (e.g. Roach, 1938, 1939).

The more recent work on the control of plant diseases by chemotherapy has been reviewed by Stoddard & Dimond (1949), Horsfall, Dimond, Stoddard & Chapman (1949), Horsfall & Dimond (1951). The main workers in this field in the United States have been Horsfall and his co-workers at the Connecticut Agricultural Station and Howard and his colleagues at Rhode Island State College. Much of their work has been concerned with systemic diseases such as the vascular wilts for which other methods of control are not available. Encouraging results have been obtained in the control of Dutch elm disease, *Fusarium* wilt of carnations, *Verticillium* wilt of maple and *Fusarium* wilt of tomatoes. Compounds used include inorganic salts, sulphonamide derivatives, hydroquinone, benzoic acid, phenols, 8-quinolinol benzoate, azo-derivatives and antibiotics. McNew & Sundholm (1949) tested 4-nitrosopyrazoles against *Alternaria solani* on tomatoes and found that introduction of certain of these substances into leaves significantly reduced the incidence of disease.

New systemic fungicides are usually sought by carrying out a routine examination, using suitable 'screening' tests, of a wide range of synthetic compounds. If by such means a compound of promise is found various derivatives and compounds of similar structure are prepared and tested. This empirical line of approach has led to the discovery of systemic fungicidal action in a number of compounds.

Many factors can determine whether or not a particular chemical can show activity as a systemic fungicide. Thus, the compound might not be taken up by the plant or transported within the plant. Further, it may be modified chemically or broken down completely under the influence of plant enzymes. Again, the chemical might be damaging to plant tissues and so unable to demonstrate any inherent capacity to act as a systemic fungicide. The biological result obtained with any chemical in a 'screening test' represents the net effects of many factors which operate under the conditions of that particular test. Any protection achieved, however, may be entirely different with different fungi and different host plants.

In this country Crowdy & Wain (1951) have studied the systemic fungicidal activity of certain aryloxyalkylcarboxylic acids, compounds which may be translocated within the plant and which can possess fungicidal properties. It was known that plant growth-regulating activity in such compounds was associated with certain structural requirements (Wain, 1951), and it was therefore possible to select compounds which had negligible effects on plant growth. Using young bean seedlings as host plant and *Botrytis cinerea* or *B. fabae* as the

pathogen, significant protection was achieved by treatment with low concentrations of aryloxy acids through the roots. It should be emphasized, however, that the systemic fungicidal protection demonstrated, and that found by Fawcett (1951) in similar experiments cannot be definitely ascribed to the fungicidal performance of the applied chemical as such within the plant. The substance may be changed in the plant to a compound of higher fungicidal value or alternatively, this introduction of a foreign molecule into the plant may lead to biochemical changes which ultimately result in the conferment of a degree of resistance. It is perhaps significant that neither Horsfall and his co-workers (Horsfall *et al.* 1949) nor Fawcett and myself have been able to find correlation between direct and systemic fungicidal action towards the same pathogen.

The work of Brian and his colleagues illustrates another line of approach to systemic fungicides. These workers studied griseofulvin, an antibiotic which is produced in culture by *Penicillium nigricans*, a fungus widely distributed in soils. It was logical to suppose that if this antibiotic is produced in the soil, it might enter plants through their roots. These considerations led to the examination of griseofulvin as a systemic fungicide and, as we shall see in a later paper, some very encouraging results have been obtained.

It is interesting to consider the natural resistance of plants to disease in relation to systemic fungicidal action. Now in nature, most plants are resistant to most pathogens—resistance is the rule rather than the exception. Such resistance can sometimes be related to morphological characteristics such as hairy leaves or a thick or waxy leaf epidermis. Again, initial attack by the fungus may lead to a rapid death of cells which then form a barrier through which the fungus cannot penetrate. There is, however, evidence that in certain cases, natural resistance may have a chemical basis.

First, the food supply available to the pathogen from the host plant may be unsuitable. Certain fungi do have very specific food requirements—for example *P. glaucum* utilizes *dextro*- tartaric acid more readily than the *laevo*- form. Resistance, then, may well arise in cases where there is a high degree of specialization in the type of food required by the parasite. Secondly, a resistance to infection may be associated with the presence of a fungicidal or fungistatic chemical within the plant. Amongst the examples which have been cited to illustrate this are the presence of protocatechuic acid in the scales of onions resistant to smudge (Walker & Link, 1935), phenolic substances in wheat varieties resistant to rust (Newton & Anderson, 1929) and linamarine (a cyanogenetic glucoside) in varieties of flax showing resistance to *Fusarium* wilt (Reynolds, 1931). The work on tomatin at the U.S.D.A., Beltsville, is also interesting in this connexion. Tomatin is an alkaloidal glycoside which is present in tomato plants. The compound possesses fungistatic activity towards the *Fusarium* species which cause wilt diseases in tomatoes, peas and cabbage (Irving, Fontaine & Doolittle, 1946). Although tomatin is present in varieties of tomato which are susceptible to *F. oxysporum* f. *lycopersici* as well as in resistant varieties, it has been shown that in the former, tomatin gradually disappears following invasion by the fungus. In resistant plants, however, even when badly infected, the tomatin level is apparently not reduced. Irving (1947) has suggested that tomatin exerts a protective function, but that only those varieties which can produce the substance at a rate sufficient to maintain a protective level in the plant can show resistance. It may be of interest to record that Fawcett has tested tomatin as a systemic fungicide in the bean test. No significant activity was shown against *Botrytis fabae* in this test and the compound was decidedly phytotoxic when supplied to the roots at 20 p.p.m.

The possibility that natural resistance may be due to the presence of specific chemicals within the plant indicates a useful line of approach to systemic fungicides, i.e. an examination of particular compounds known to exist in certain higher plants. Such compounds might at least be expected to remain stable within the plant, and some might well be found to confer fungicidal protection against certain pathogens. Another line of approach, the examination as systemic fungicides of extracts of various plants which are resistant to specific pathogenic fungi, might also be considered. Such work could be followed later, if warranted, by

a chemical investigation of the extracts to identify any compounds present possessing systemic fungicidal activity.

Already, extracts of many kinds of plants have been examined for antibacterial and antifungal activity. In one series of investigations (Scott, McKay, Schaffer & Fontaine, 1949) approximately 40% of the extracts from the eighty plants tested exhibited antibacterial or antifungal activity or both (see also Osborn, 1943; Huddleson, DuFrain, Barrons & Giefel, 1944; Lucas & Lewis, 1944; Seegal & Holden, 1945). Bruckner, McKay, Schaffer & Fontaine (1949) showed that the leaves, stems and roots of the sweet potato contain substances which inhibit the growth of two species of wilt fungi as well as certain bacteria. In some cases, active constituents have been isolated in crystalline form, for example the tomatin already referred to and the substance tentatively identified as the sulphoxide of diallyl disulphide obtained from garlic (Cavallito, Buck & Suter, 1945).

Much work of this kind remains to be done in relation to systemic fungicides. The problems in this field of study are undoubtedly complex and varied, but in my opinion vigorous attack both from the chemical and biological aspects is warranted—and here, of course, the closest team work is essential.

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TECHNIQUES IN THE BIOASSAY OF SYSTEMIC FUNGICIDES

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In the limited time at my disposal, it would be out of the question to attempt an exhaustive survey of the techniques used in testing compounds for the internal treatment of plant diseases, particularly as some of these tests will be considered by the later speakers. This talk will, in fact, be devoted largely to describing the test which we have developed at Long Ashton, and I will try to indicate some of the difficulties which we have encountered. However, before I deal with the particulars, I will try to outline some general principles.

The term 'chemotherapy' has been used in the U.S.A. to describe the treatment of plant diseases with chemicals which, are, to a greater or less extent, mobile within the host. The term is used in contrast to protection, where the action takes place outside the host. In this sense, chemotherapy is simply a statement of an observed effect and gives no indication of the method by which the disease is reduced. A 'systemic fungicide', on the other hand, may be described as a particular type of chemotherapeutant, and is a term which gives a precise description of the behaviour and action of the chemical under discussion. I think that Prof. Wain and I were entitled to use a term of this precision in the work we have published since we deliberately studied a group of compounds which were thought to be systemically distributed within the higher plants, and found that within that group the more fungicidal members were the ones which were active in reducing disease. It is, however, tempting to extend the term by analogy to any compounds which have a comparable effect even if one has no evidence that they can be translocated as fungicides. In these cases the more general term chemotherapeutant is probably more safely used.

The effect of a systemically distributed chemical on a plant disease is a very complicated biological system to study, and I think it is safe to say that most of the work has been confined to applying a chemical at one end of the system and attempting to measure the effect on the disease at the other. In these circumstances the assessment of the disease and the correct interpretation of the results is of major importance.

In most of the observations on systemic fungicides, the assumption has been made that treatment will not prevent the establishment of infection but that it will retard the development of a disease which has been established. In these circumstances disease assessment is concerned with measuring the rate of disease development: this is usually effected by assessing the extent to which a disease has developed a definite time after its establishment. The actual criteria used in assessment, of course, vary. Direct lesion measurement and assessment of area damaged have been used with chocolate spot of bean, an arbitrary scaling of damage with Dutch elm disease and *Botrytis* on lettuce, an arbitrary assessment of vascular discoloration in certain vascular diseases and a count of visible lesions in the case of early blight of tomato.

The chemicals have usually been applied to the roots of the host plant, and this method

seems to be the easiest to handle experimentally and to give the best distribution of the chemical. Success has also been reported with compounds sprayed on to the leaves, injected into the leaves or stems or smeared on the stem in lanoline solution.

I think that some of the difficulties which are likely to crop up in testing for systemic fungicidal activity are best illustrated by a description of our own work. In routine testing the beans are raised from seed in pots, lifted and transferred to small jars containing solutions of the chemicals for varying periods, up to 3 weeks, prior to inoculation. After treatment they are inoculated by spraying with a spore suspension of *B. fabae* till the plants are dripping. No attempt is made to standardize the inoculum from one trial to the next, but within a trial all the plants are sprayed to run-off with the same spore suspension thus ensuring as uniform infection as possible. After inoculation the plants are held in a saturated atmosphere for 2-3 days at a temperature of about 70° F. The extent of the disease may be estimated on the leaves, flowers or pods.

Three methods of estimating the disease on the leaves have been used: (a) grading on an arbitrary scale; (b) estimating percentage area damaged by comparison with standard diagrams; (c) measuring diameters of individual lesions.

The first two are eye estimates of gross damage and differ only in the standards of measurements used: the second is preferable since the standard diagrams are available to provide continuity through the work. Both suffer from two disadvantages: (i) they are purely subjective estimates, (ii) the final result will be markedly affected by the actual rate of infection as well as by the size of the individual lesions.

Both estimates are therefore dependent on the uniformity of the method of inoculation, and although this has been made as uniform as possible, the leaves are, in practice, difficult to wet, with the result that the distribution of lesions tends to be erratic. Measuring the diameters of individual discrete lesions avoids both these snags to some extent. If one assumes that the ideal lesion is circular, the square of the diameter gives an estimate of the area of each lesion, and from this figure an estimate of the reduction of area diseased following treatment can be made by comparison with the controls. This estimate assumes that the actual number of lesions formed throughout is uniform and therefore smooths out irregularities in the inoculation technique. It also obliterates any effect the compound may have in preventing lesion formation and may, therefore, tend to underestimate the effectiveness of treatment. There is a linear relationship between estimates of damage made by these methods.

Flower damage has been assessed as the percentage of dead flowers per inflorescence.

Disease assessment is complicated by the fact that there is a marked gradient on the affected plants: fewer lesions are formed on the youngest leaves, and the lesions that are formed are smaller. Flowers on the youngest inflorescences are also more resistant to infection. On the leaves the effect of this gradient is very marked on the three youngest leaves, but there is less variation in the centre of the plant and it would, therefore, be possible to avoid much of the variation resulting from this effect by confining assessment to the centrally placed leaves. There is, however, a serious objection to this course, since certain compounds appear to travel preferentially to the most actively growing parts of the plant and this effect would be lost if assessment avoided the young tissues.

This effect is very strikingly illustrated when assessing the disease on flowering or fruiting beans treated with phenoxy acids. In one trial with pentachlorophenoxyisobutyric acid the treated plants showed a significantly higher level of disease than the controls on their leaves and at the same time a significant reduction in flower damage. The same effect has been recorded with plants in fruit when comparing spotting on the leaves and the pods: the leaves of plants treated with this compound were damaged to the same extent as the controls while the pods showed only about half of the damage recorded on the untreated plants.

A complication of a similar type is sometimes seen when the concentration of the chemicals is varied, for example manganese dinicotine salicylate at a concentration of 1 p.p.m. produces a marked effect on the 2nd and 4th leaves, but its effect is not appreciable

on the top leaf where the natural resistance of the host is high. At the higher concentration of 10 p.p.m., on the other hand, there is a definite reduction in lesion diameter on the top leaf but the effect of treatment becomes progressively less on the older leaves. This suggests to us that the most actively growing leaves are less liable to damage following treatment than those which are less active and that, therefore, here the higher concentration produces a reduction in disease; lower on the plant lesion diameter reflects both the fungicidal activity of the compound, reducing the spread of the disease, and the phytotoxicity of the treatment, which lowers the resistance of the host. The phytotoxic effect becomes more marked on the older and less vigorous leaves with the result that the effect of treatment is obscured. This behaviour parallels that recorded above with the phenoxy acids, and reflects the effect of age and loss of vigour of the various plant parts on the development of the disease.

In closing it is probably worthwhile considering briefly some of the results which we have obtained with compounds other than the phenoxy acids. There is some evidence that the derivatives of benzoic acid are mobile within the plant, and it is not surprising that some of these are active in reducing chocolate spot; for example, salicylic acid and certain of its derivatives, and *p*-aminobenzoic acid.

The naphthoxy acids are also known for their growth-promoting activity and these are also active to a certain extent, though apparently less effective than the phenoxy acids.

Finally, a selection of miscellaneous compounds which have been reported in America as reducing various diseases have also reduced chocolate spot of bean. These include certain hydroxyquinoline derivatives, an azo dye, helione A (2-4-diamino-5-methyl-azobenzene hydrochloride) and some thiourea derivatives.

ANTIBIOTICS AS SYSTEMIC FUNGICIDES AND BACTERICIDES

By P. W. BRIAN, *Butterwick Research Laboratories, The Frythe, Welwyn, Herts*

We have not done a lot, as yet, about systemic fungicides at the Butterwick Research Laboratories, but what we have done has been based on an approach rather different from that of other workers, and may for that reason be of some interest to you.

One of our main interests for some years has been the production of antibiotics by fungi. Though the capacity to produce antibiotics is not by any means restricted to soil fungi, it is true that a high proportion of the saprophytic soil inhabitants do produce antibiotics in artificial culture. There has been considerable speculation whether this capability is of natural significance; it is tempting to associate it with the well-known antagonism between certain soil saprophytes and some root pathogens. As yet, such speculations are backed by little solid experimental evidence, the main gap in our knowledge being that we do not know whether micro-organisms can and do produce antibiotics under soil conditions. Several fungi have been shown to produce antibiotics in sterile soil to which a suitable energy has been added. Hessayon, at Manchester University, has shown that the fungus *Trichothecium roseum* will produce an antibiotic in sterile soil without an added energy source, and my colleague Miss Wright has shown the same for the fungus *Trichoderma viride* and for several species of *Penicillium*. But so far no one has demonstrated the production of an antibiotic in soil that has not been at least partially sterilized. I believe this to be largely because the right technique has not yet been found, the difficulty being to detect the small quantities in the restricted *loci* that would be involved. I see no *a priori* reason why they should not be formed, since after all it is now well established that vitamins are produced in soil as a result of microbial metabolism, and these are not widely dissimilar molecules.

It is now a fairly general view that if antibiotics are produced in soil they will be produced most particularly in those areas where microbial activity is most pronounced, that is in the vicinity of particles of decomposable organic material and in the rhizosphere. Following this line of thought, it occurred to us, as it has also occurred to Krassilnikov (1949), that

antibiotics produced in the rhizosphere might well pass into the roots of plants and thence be translocated into other organs. If this does take place under natural conditions, of course, all sorts of implications arise, but this is not the time to discuss them.

We carried out a number of experiments based on this approach (Brian, Wright, Stubbs & Way, 1951). First, we sought to demonstrate uptake of an antibiotic by a plant, through its roots, and translocation of the antibiotic to the shoot. This is not necessarily an easy matter. We were lucky in that we succeeded in doing it with the first antibiotic tested—the substance known as griseofulvin. But, if I may say so without seeming unduly immodest, it was not entirely a matter of luck but depended also on an intelligent selection of the antibiotic to study. Let me explain the considerations that influenced us.

The antibiotic should be non-phytotoxic. Miss Wright has examined a large number of antibiotics for phytotoxicity. Of those we have tested, penicillin has been least phytotoxic, followed by streptomycin, and that followed by griseofulvin. All the remainder were quite highly phytotoxic and, since we wanted to use relatively high concentrations to make detection in the plant as easy as possible, they were rejected for preliminary work. The antibiotic should be stable in aqueous solution. Many of those we have handled are stable only in acid solution. Penicillin is stable only near neutrality. Among the most stable is griseofulvin, though it is inactivated biologically in some soils. Finally, the antibiotic should be susceptible of assay in low concentrations and as specifically as possible. Griseofulvin is particularly suitable from this point of view: a concentration of $0.1 \mu\text{g./ml.}$ can easily be detected; 0.1 ml. of material is sufficient, thus $0.01 \mu\text{g.}$ can be detected; the assay is specific, as far as our experience goes, to griseofulvin and some of its derivatives. Thus griseofulvin was a fairly obvious choice: it has some disadvantages, one being its low solubility in water—not much more than 30 mg./l. in the cold.

Perhaps I had better remind you of some of the properties of griseofulvin. It is a metabolic product of three species of *Penicillium*—*P. griseofulvum*, which is apparently a rare fungus and perhaps only a variant of the next species I am going to mention, *P. patulum*, which is not uncommon, and by *P. nigricans* which is very commonly found in soil, particularly in acid soils. Griseofulvin was first isolated in Prof. Raistrick's department at the London School of Hygiene, and Oxford, Raistrick & Simonart published a tentative molecular formula in 1939. In 1946 we isolated from *P. nigricans* a substance that we called 'curling factor', which we subsequently showed to be identical with griseofulvin, whose biological activity until then was unsuspected. Recently a group of my colleagues (Grove, Ismay, MacMillan, Mulholland & Rogers, 1951) have established with a fair degree of certainty a molecular formula differing in several respects from that of Oxford, Raistrick & Simonart. Griseofulvin is not a fungicide. It does not prevent germination of fungus spores but it has a remarkable effect on hyphal development. This is conveniently seen in its effect on germinating conidia of *Botrytis allii*. High concentrations ($c. 10 \mu\text{g./ml.}$) cause stunting of hyphae, excessive and abnormal branching, hyphal distortion and loss of apical dominance. The effect is reminiscent by analogy with the effect of some auxin analogues on higher plants. Low concentrations ($c. 0.1 \mu\text{g./ml.}$) produce a regular spiral waving of the hyphae. Our bioassay for griseofulvin is based on observation of these effects. Not all fungi are as sensitive to griseofulvin as *B. allii*. *Pythium* spp. and *Phytophthora* spp. are unaffected. *Trichoderma viride* is only affected slightly. But many are sensitive, and growth in such cases is reduced to a negligible level by appropriate concentrations of griseofulvin. We have at present very few clues to the mode of action of griseofulvin and those that we have are rather tenuous.

The technique we adopted for preliminary experiments was based on an ingenious method developed by Frohberger (1948) in connexion with studies on systemic insecticides. It is so simple as to be almost crude. The apparatus consists of a tall beaker, a Perspex cylinder, a piece of surgical gauze and an elastic band. The gauze is tied over one end of the Perspex cylinder, and wheat seeds are placed on the gauze; this is then placed in the beaker and a mineral nutrient solution (Pfeffer's solution, modified) poured in to just below the gauze. The assembled apparatus is then placed in a humidity chamber (bell-jar) until the seeds have

germinated. Roots grow down into the nutrient and the plants grow vigorously if the nutrient is periodically replaced. When the seedling is 2-3 in. high any substance whose uptake is to be studied is added to the nutrient solution. The procedure from this point is to see if the substance can be detected in the guttation drops, which are readily formed if the cultures are placed in conditions of high humidity overnight.

We found that griseofulvin could be detected in the guttation drops in 2-3 days, if added to the culture solution at concentrations of 2-50 $\mu\text{g./ml.}$ It is preferable to work with concentrations of the order of 2-5 $\mu\text{g./ml.}$ as higher concentrations are phytotoxic, causing tip-burn of the leaves and peculiar lateral tumours on the primary roots, as well as reducing the growth rate of shoot and root. Thus griseofulvin was shown to be taken up by the wheat seedlings and translocated to the leaf-tips. It is possible, of course, that the material appearing in the guttation-drops is not griseofulvin but some derivative showing similar biological activity. It is not possible at present to say whether this is so or not. But we do feel fairly certain that any chemical modification of the griseofulvin molecule that may have occurred is in any event only slight. Miss Stokes is at present using this guttation-drop technique to study the effect of environmental conditions on the uptake and translocation of griseofulvin, considering such factors as temperature, humidity, light, concentration and composition of nutrient and so forth. I do not propose to describe her results, which are incomplete, except to say that weather conditions do profoundly affect the rate of uptake of griseofulvin; whereas in summer in a greenhouse griseofulvin reaches the guttation drops in 2 days, it may take 7 days or even longer in winter. This is worth bearing in mind. It indicates that whereas systemic fungicides may not be affected by such hazards as leaching by rain, their efficiency may be profoundly affected by weather in other ways.

I must not digress however. Having established the uptake of griseofulvin by wheat plants we turned to other species of plant. In the case of plants which do not produce guttation drops we found it possible to detect griseofulvin in the leaves by grinding up leaf tissue with a little distilled water and assaying the filtrate from such a preparation. Uptake of griseofulvin turned out to be general. Knowing its antifungal nature we were naturally led to consider whether uptake of griseofulvin by plants conferred on them resistance to attack by pathogenic fungi. In a preliminary experiment we found significant control of *Botrytis cinerea* on lettuce. We were sufficiently encouraged by this result to enlist the collaboration of Dr Stubbs who had already developed more elegant and quantitative methods of estimating protective efficiency. He will be describing his results to you shortly; all I need say now is that he found that systemically applied griseofulvin gave excellent control of *Alternaria solani* attack on tomatoes. Since then we have obtained good control of *Botrytis fabae* on broad bean, and Mr F. T. Last of Rothamsted has demonstrated control of *Erysiphe graminis* on barley growing in water culture. I am indebted to him for permission to quote some of his results. His experiments have shown clearly that the degree of protection afforded by griseofulvin decreases in time. We have other evidence that this is so, but whether it is due to destruction of the griseofulvin in the leaf tissue, translocation to other parts of the plant or to some other cause, we do not yet know.

Such experience as we have had indicates that griseofulvin is a fairly effective systemic fungicide. But we have abundant evidence that much depends on the conditions of test. We are at present trying to standardize these and then hope to secure some insight into the kind of molecule that makes a good systemic fungicide by comparing a wide range of griseofulvin derivatives. Standardization of methods of test is also necessary if results obtained in different laboratories are to be validly compared. The ability to make such comparisons is particularly valuable now, while we are at the beginning of investigation of a new method of disease control.

Griseofulvin is not the only antibiotic which has shown promise for systemic treatment of plant disease. In 1944, Brown & Boyle showed that if penicillin were applied to crown gall tumours the bacteria throughout the gall were killed in a way which strongly suggested that the penicillin penetrated deeply into the gall tissue. More recently, Blanchard & Diller

(1951) showed that aureomycin was taken up by the roots of *Phaseolus lunatus* and appeared in the leaves, and Blanchard (1951) followed this up by showing that aureomycin, like penicillin, was effective in the chemotherapeutic treatment of crown gall in tomatoes. More recently still, Mitchell, Zaumeyer & Anderson (1952) have shown that streptomycin applied in lanolin to stems of *Phaseolus* confers resistance to the leaves against bacterial blight caused by *Pseudomonas medicaginis*.

I should like now to return more closely to my original theme. We have shown that if griseofulvin is added to culture solutions in which plants are growing, it appears in the aerial parts of the plant. If it is added to soil in which plants are growing it still appears in the plant shoots but very much less of a given quantity applied appears in the plant. My colleague E. G. Jefferys has shown that griseofulvin is microbiologically degraded in fertile soils with some rapidity. So it appears to me that even if griseofulvin could be produced as cheaply as copper sulphate it might still be uneconomic to apply it to soil. An alternative is application to foliage, but this is almost certainly a less effective method of entry to the plant. How much more satisfactory if the appropriate organism could be induced to synthesize it in the rhizosphere of the plants which it is desired to protect. This seems to me to be a line of investigation that would be well worth following, beset with pitfalls though it is.

We have carried out a number of experiments which bear somewhat inconclusively on this point. An inoculum was prepared by growing *Penicillium nigricans* on a sterile soil + cornmeal mixture. This was added to sterile soil and to ordinary soil and wheat plants were grown in it. Griseofulvin undoubtedly appeared in the guttation drops, more particularly in the sterile soil series. This looked promising, but it was found that griseofulvin was preformed in the soil + cornmeal inoculum and that this griseofulvin could account for much if not all of that appearing in the wheat plants and guttation drops. As I said, these experiments are at best inconclusive but I believe that some such approach might yield valuable results.

I should like to turn now, if I may, to another aspect of systemic treatment of plant disease, where again our work on fungal metabolism has some bearing. You will recall that a number of American phytopathologists conceive one kind of chemotherapeutic treatment of plant disease as 'antidoting the toxins produced by the parasitic fungus'. For example, Howard (1941) reduced the severity of attack by *Phytophthora cactorum* on maple shoots by treatment of the plants with diaminoazobenzene hydrochloride. Experimental evidence was produced that the growth of the parasitic fungus was unaffected but that the toxic effects of some substance produced by it were reduced. I do not think the evidence of toxin inactivation was unambiguous, but we can accept it for the moment. Horsfall & Zentmyer (1941) obtained similar results with the same compound in Dutch elm disease. Now we know very little in general about phytotoxic substances produced by plant-parasitic fungi, but two have been studied in some detail in pure form. Perhaps the clearest examples of plant diseases some of whose symptoms are toxigenically induced are the *Fusarium* wilts. From *F. lycopersici*, Prof. Gäumann and Prof. Plattner and their colleagues in Zurich have obtained the substance lycomarasmin. This substance is toxic to plants and can reproduce in tomatoes many of the symptoms of *Fusarium* wilt. A number of 'antidotes' to lycomarasmin are known; one of them is the well-known peptide glutathione. It has been suggested that if it were not so expensive glutathione might be used for systemic treatment of tomato plants suffering from *Fusarium* wilt. But, it is not known how many strains of *F. lycopersici* produce lycomarasmin. From the published work it appears that the Swiss workers have studied only two strains. One, which produces lycomarasmin in considerable yield is almost non-pathogenic but another, highly pathogenic, produces little or no lycomarasmin. It seems that other toxic substances besides lycomarasmin are produced by some strains, and unless glutathione were an antidote to these as well, glutathione chemotherapy would be specific to certain strains of *F. lycopersici* only.

Another fungal toxin that has been studied in pure form is alternaric acid. This was obtained in our laboratories from two strains of *Alternaria solani*. It is capable of reproducing 'synthetically' many of the disease symptoms produced in *A. solani* attack. But these two

strains of *A. solani* from which we first isolated it were almost non-pathogenic, and a number of highly pathogenic strains of *A. solani* which we have since investigated produce no alternaric acid. A pathogenic strain studied by Pound & Stahmann (1951) at Wisconsin does produce alternaric acid. So again it seems that an 'antidote' to alternaric acid would only alleviate disease symptoms in the case of attack by some strains of *A. solani*. I have mentioned this point, not to deny the validity of the concept of toxin-inactivation, but to show how complex the systems involved are likely to be, and to suggest that reliable disease control by such methods may be a difficult matter.

In conclusion, I should like to return once more to systemic protectants of the kind we have been discussing to-day. Most workers who have studied their activity take the view that there is little correlation between *in vitro* fungicidal or fungistatic activity and systemic activity. You have seen this both with griseofulvin and the aryloxyaliphatic acids discussed by Prof. Wain and Mr Crowdy and the same is true of the compounds used by American workers. This is perhaps not surprising. The inside of a plant is an environment of intense chemical activity. A plant is quite capable of chemically changing foreign substances introduced into it. Workers at the Boyce-Thompson Institute, for example, have shown that potato tubers, placed in an atmosphere of ethylene chlorhydrin, store away enormous amounts of the chlorhydrin as a complex glucoside. Similarly, our systemic fungicide may undergo reactions leading to something more antifungally active, or less antifungally active. Again its activity may only appear when fungal invasion calls forth certain responses from the host tissues. The possibilities are legion; the point to bear in mind is that here we have a much more complex system than with the more conventional layer of protective fungicide on a leaf surface.

Another point to which I should like to draw your attention is a possible extra advantage that might be derived if it were found possible in practice to introduce systemic fungicides into plants through the root system. Newhook (1951) and Wood (1951) have very elegantly demonstrated that the antagonism between saprophytes and parasites so well known in soil is paralleled by a similar antagonism between saprophytic micro-organisms of the leaf surface and invading pathogens. Foliage application of a fungicide probably kills saprophytes and pathogens more or less impartially. A systemic fungicide would probably only affect parasites penetrating the epidermis—certainly we have found that *Botrytis fabae* spores germinate and germ-tubes readily penetrate the epidermis of bean leaves protected systemically by griseofulvin—and would leave the saprophytes unharmed. Thus biological control would be brought to the aid of the systemic fungicide. I believe that all chemical approaches to disease control should envisage such a selective approach.

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THE EVALUATION OF SYSTEMIC FUNGICIDES BY MEANS OF *ALTERNARIA SOLANI* ON TOMATO

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I wish to deal briefly with the method we use at the Hawthorndale Laboratories for investigating the systemic fungicidal activity of griseofulvin and other materials. Our work on systemic fungicides has necessarily depended on our working out a suitable and reliable technique.

Our first problem was, of course, to find a suitable pathogen and host. This was soon solved. We have an excellent culture collection at Hawthorndale, and various diseases are in regular use for the greenhouse evaluation of foliage fungicides. After some thought we selected *Alternaria solani* on tomato for this work for two main reasons. One was that the disease develops rapidly so that only a short period of protection is required. The other was that in preliminary foliage spray tests, griseofulvin gave good control of the disease.

Before describing the methods we used for applying the chemical, let me clear the ground by telling you briefly how we inoculated the host plants and estimated the amount of disease. The method was based on that of McCallan & Wellman (1943) and, with minor modifications, was standard in all tests.

Inoculation was by means of an aqueous suspension of spores of *A. solani* at a concentration of 25,000–30,000 spores per ml. We used the tomato variety Best of All as the host and, while holding the plants in the hand, sprayed the suspension uniformly on to the lower surface of the leaves, using a simple right-angle jet atomizer. The plants were then kept at high humidity for 24 hr. before returning them to the greenhouse. About 3 days after inoculation small discrete, dark-coloured lesions develop on the leaves. These spots, fortunately enough, are all about the same size so that the amount of disease can be estimated merely by counting them. There is no need for elaborate size determinations. The number of lesions on the treated plants expressed as a percentage of those on the control plants gives a figure for the percentage disease.

To apply the chemical we tested two main methods, namely root and leaf absorption. Injection techniques were not tested as it was felt that these were not suitable for use with herbaceous plants.

In our early root-absorption tests the plants were grown either in water or in sand culture and we added the chemical to the nutrient medium. We soon found, however, that we had to discard the water-culture method owing to cultural and other difficulties. The sand-culture method, on the other hand, turned out to be very satisfactory and we developed a simple form of it for routine sorting work.

The original sand culture involved growing tomato plants to a height of 6 in. in wax cartons containing about 600 g. of sand. Treated plants were watered daily for 4 days with 50 ml. of a solution or a dispersion of the test chemical while the control plants received tap water. In addition, all plants were also watered with nutrient solution. Three replicate plants were used for each treatment. Inoculation was done on the fifth day. Subsequently the lesions were counted on the twenty-five main leaflets of the lower five leaves of each plant. There were, on the average, about 1000 lesions on each control plant.

Typical results are given in Table 1.

You will notice that both the concentrations of griseofulvin used by us were much higher than those tested by Dr Brian. We selected this range after some preliminary tests, and it has proved to be very satisfactory. Both concentrations were effective against *A. solani*. Neither caused any visible damage or retardation of growth. A point of some interest with the 0.05 % griseofulvin was that, although this treatment consistently gave 99–100 % disease control, close inspection showed that small abortive infection points were sometimes left.

The nitrosopyrazoles were markedly less active than griseofulvin. We included these compounds so that comparison could be made with the leaf-immersion technique used by McNew & Sundholm (1949). These authors reported the two chemicals listed above as giving 31 and 44% control of *A. solani*, so that in this case similar levels of control were given by widely different methods of application. You will have noticed, however, that the relative efficiencies of the two chemicals were reversed. This was not unexpected since McNew & Sundholm found that with 1-*p*-sulphamylphenyl-3:5-dimethyl-4-nitrosopyrazole the lesions were slow to appear and we made our lesion counts sooner after inoculation than they did. Incidentally, we found that the nitrosopyrazoles restricted the growth of the tomato plants and that, in one case at least, 1-*p*-sulphamylphenyl-3:5-dimethyl-4-nitrosopyrazole, applied to the roots, caused slight burning of the foliage.

TABLE 1. *Evaluation of systemic fungicides (Alternaria solani). Original sand-culture method*

Treatment		% disease control
Chemical	%	
Griseofulvin	0.05	99-100
	0.01	74
1- <i>p</i> -Sulphamylphenyl-3:5-dimethyl-4-nitrosopyrazole	0.05	48
1-Phenyl-3:5-dimethyl-4-nitrosopyrazole	0.05	23

Having demonstrated the usefulness of this method we set out to simplify it for the screening of large numbers of chemicals. After considering greenhouse space and other factors, the most important of them being the need to reduce the amount of labour in plant production and disease estimation we standardized a method in which we now use smaller tomato plants, grown in small wax cartons containing sand. We apply the chemical as before, but at a correspondingly reduced rate, to each of two replicate plants. Disease estimations are restricted to the lowermost normal leaf, and give a count of 100 to 200 lesions on the control plant.

Typical results with this method are given in Table 2.

TABLE 2. *Evaluation of systemic fungicides (Alternaria solani). Simplified sand-culture method*

Treatment		% disease control
Chemical	%	
Griseofulvin	0.05	99-100
1- <i>p</i> -Sulphamylphenyl-3:5-dimethyl-4-nitrosopyrazole	0.05	54
2:4:6-Trichlorophenoxyacetic acid	0.05	31
	0.01	7

Again griseofulvin at 0.05% gave almost complete disease control whilst the nitrosopyrazole behaved much as it did before. 2:4:6-Trichlorophenoxyacetic acid at 0.05% gave similar control of *A. solani* to that reported by Crowdy & Wain (1950) for *Botrytis* spp. on broad bean. At 0.01%, however, it gave only slight control of *Alternaria solani*.

Another phase of this work has been to determine the persistence of griseofulvin within the plant. To study this effect the plants were repotted in fresh sand at the end of the pretreatment period and inoculated with *A. solani* at various intervals. We found that griseofulvin remains fully active for 4 days but after a week it is only half as effective. This rather rapid loss of activity did not surprise us since the chemical is operating within the complex dynamic system of the plant. It does, however, emphasize that systemic fungicides are not necessarily more persistent than conventional spray applications.

The uptake of griseofulvin through the roots of tomato plants has also been studied at Hawthorndale using John Innes no. 1 potting compost in place of sand. Results have been similar to those obtained in sand culture. The compost apparently has no immediate effect on the systemic activity of griseofulvin.

We have naturally given some attention to the absorption of chemical through the leaves. It seemed to us that the two major difficulties were likely to be a localized pattern of distribution and limitations in the supply of chemical available for absorption. McNew & Sundholm (1949) had solved the problem of supply by using a leaf-immersion technique. Our approach was to spray a number of leaves and subsequently carry out disease estimates on leaves which had been shielded from the spray. In some trials we sprayed only the two lowermost leaves. In others we sprayed the entire plant with the exception of a single leaf which was shielded by a polythene envelope. With both methods it was necessary to delay inoculation for at least 4 days after spraying.

Development of this method is not yet complete. So far the indications are that, with griseofulvin, the leaf application is less effective than root absorption. Results are also very variable. We obtained, for example, better protection in summer than in winter, confirming Brian's finding that weather affects systemic activity.

There is little doubt in my mind that, on the evidence at present published, root absorption is a more effective way of evaluating systemic fungicides than is leaf absorption. This, however, may not necessarily be the case with all chemicals or disease, and both methods should therefore be studied.

In conclusion I would like to say a few words about the selection of chemicals for testing as systemic fungicides. *In vitro* sorting tests do not appear to be applicable in this case since they can give no indication of the ability of the chemical to enter a plant or of its activity within a living system. For this reason, although we have yet to demonstrate high systemic activity in chemicals showing no activity as foliage fungicides this is a possibility that cannot be precluded. Certainly it is not difficult to visualize the transformation within the plant of an inactive chemical into an effective fungicide. At present, therefore, the only alternative to testing as many chemicals as possible seems to be the method of intelligent selection as described by Crowdy and by Brian.

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ROOT INJURY AS A FACTOR IN THE ASSESSMENT OF CHEMOTHERAPEUTANTS BY THE TOMATO *FUSARIUM* WILT TEST

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Studies of the tomato *Fusarium* wilt test for screening chemotherapeutants were made by the writer at the Connecticut Agricultural Experiment Station, U.S.A., during 1951. The test compounds were applied on ten successive days to the roots of tomato plants growing in sand. The plants were then uprooted, their roots washed and dipped in a bud-cell suspension of *F. oxysporum lycopersici* and the plants re-potted. A standardized assessment of wilt and vascular discoloration was made some 21 days later, by which time the control plants were usually severely diseased. Several compounds greatly reduced disease severity.

After observations that certain of these compounds caused root injury, tests were made of other modes of root injury prior to inoculation to determine whether this influenced subsequent wilting. The methods used were (a) dipping the roots in water at 55° C. for 30 sec.; (b) periodic pruning of the roots; (c) treatment with compounds known to cause root injury. All three treatments greatly reduced subsequent wilting. Several compounds unlikely to be translocated in the plant (e.g. hydrogen peroxide) were highly effective in reducing disease.

Further tests showed that the effect was unlikely to be associated with fungal penetration through the injured roots but was probably caused by an induced resistance of the upper parts of the roots or of the lower stems. It was further noted that the metabolism of the injured plants had been greatly altered, tending to an accumulation of soluble carbohydrates in the aerial parts. It appeared likely that the effects of certain compounds lay in their effect on host resistance and not in systemic fungicidal action on the pathogen.

These observations emphasized the fact that the chemical treatment of plants in an attempt to obtain systemic fungicidal action might affect the host. Such effects might not necessarily be as drastic as those noted above but might, even so, play a part in any subsequent reduction in fungal invasion. The work also suggested ways in which host resistance might be further investigated.

REVIEWS

Flies of the British Isles. By C. N. COLYER and C. O. HAMMOND. Pp. 383. London and New York: Frederick Warne and Co. Ltd. 1951. 30s.

The authors say this book 'is intended to provide in simple fashion such illustrations and descriptions as will serve to give a general introduction to the British Flies, together with brief accounts of some of the more interesting life histories and habits'. It is apparent almost at a glance that this very modest aim has been greatly exceeded—the book provides a comprehensive account of all the families of British flies with some of the finest illustrations of Diptera yet published.

Compared with other orders of the Insecta the Diptera have been sadly neglected both by amateur naturalists and by the majority of professional entomologists. True there have been a few enthusiasts who fortunately have been a select band of specialists and who have laid sound foundations, but of good comprehensive accounts of British flies there has been none since that grand old standby Wingate's *Durham Diptera*. Not only then is Colyer and Hammond's book an excellent book on Diptera but it fills a long-felt want and will almost certainly attract new recruits to the study of this most important order of insects.

The book opens with a 42-page introductory chapter which simply but carefully outlines the principles of morphology and classification, and this is followed by a 2-page Key to Sections. The bulk of the book, some 275 pages, deals systematically with the suborders and families of Diptera; each family is very briefly described and further interesting biological information is given about the more important genera and species. There are fifty-five half-tone plates, forty-eight plates in colour and twenty-nine text-figures; 286 representative species are depicted, all the illustrations are good and some of the coloured ones are exquisite.

The book is beautifully produced in the best traditions of the Wayside and Woodland Series. Authors and publishers have quite manifestly done a real service to the science of entomology in Britain.

I. THOMAS

Handbuch der landwirtschaftlichen Versuchs- und Untersuchungsmethodik. Band VIII.

Der Vegetationsversuch. Die Methodik der Wasserkultur höherer Pflanzen. By WILHELM SCHROPP. Pp. xi+313 with 85+65 text-figures and photographs. Radebeul und Berlin: Neumann Verlag. 1951. 22 DM.

This volume is a new addition to the series of handbooks brought out under the auspices of the Verband Deutscher Landwirtschaftlicher Untersuchungs- und Forschungsanstalten edited by Dr Rudolf Herrmann.

Dr Schropp has had over 15 years' personal experience with water-culture methods and is thus well qualified to undertake a work of this nature. From the table of contents and the excellent index at the end, it is evident that the subject has been considered from every possible angle, and as a book of reference the reader will have little difficulty in finding the section dealing with his inquiry. The two longest of the six chapters are those concerned with technique and nutrient media respectively. In the former, detailed information is provided regarding the setting up and carrying out of experiments in water culture and tables given showing how factors such as size of container, number of plants per container, and environment may influence results. Among the more modern methods described, mention may be made of those for sterile culture, for maintaining constant temperature and humidity conditions and for growing tuber-forming plants such as the potato. In the chapter on nutrient media, the composition of over seventy complete nutrient solutions is given, dating from the

days of the pioneer workers, Sachs, Knop and Nobbe. The majority of these solutions will be unfamiliar to the worker of to-day and though they are of interest, as are also the pH values recorded for some of them, they are probably of little practical use at the present time. Of more value are the sections concerned with the iron supply, aeration and water relations. The suitability of different solutions for different plants and the application of the water-culture method for commercial crop growing form the subjects of two further chapters, and the volume concludes with an appendix for the diagnosis of deficiency symptoms of both major and certain trace elements in a number of agricultural crops. The photographs in the appendix (two of them coloured) are well selected and care has been taken to include examples of both mono- and dicotyledons in each case.

The survey of the literature, with author index included, has been wide, and references to the original publication are given for every authority quoted. It is, however, to be regretted, that as the writer himself states, so much of the recent literature published outside Germany has not been available to him.

K. WARINGTON

Disease in Plants. By NEIL E. STEVENS and RUSSELL B. STEVENS. Pp. xx+219, 61 text-figs. Waltham, Mass.: Chronica Botanica Co. 1952. \$4.75.

Specialization, ever narrower, is probably fundamental for the advance of plant pathology, although to the advisory pathologist the outlines of the different specialities are frequently blurred. The control of a virus disease, for example, often proves to be an entomological problem, *Botrytis* spot of tomato fruits is mistaken for insect injury, while genetical disturbances and toxic effects may simulate symptoms of parasitic disease. Also, rational control measures advocated by laboratory workers may be impossible to implement for social or economic reasons, and the adviser unable to detect the commoner cultural defects is unlikely to obtain the confidence of a grower. This essential unity of plant pathology, although widely recognized to-day, is not always apparent from the standard text-books, and it is well that biologists in general should have available a broad review, which will allow pathologists to see their chosen field in perspective.

One of the earliest, and still for its day one of the most comprehensive, attempts to meet this need was the series of one hundred and seventy-four articles contributed by an English country clergyman to the *Gardeners' Chronicle and Agricultural Gazette* during 1854-1857. The book under review is the latest. Both the Rev. M. J. Berkeley's *Vegetable Pathology* and the Stevens's *Disease in Plants* show features in common. In both there is analysis into the plant, the pathogen, and the environment. Berkeley devoted many of his earlier articles to a consideration of the plant in a state of health, and two of the preliminary illustrations of *Disease in Plants* are full-page figures of the anatomy of shoot and leaf. Subsequent chapters deal with the causal agents of disease. These are in places rather over-condensed and the omission (to which the authors draw attention) of a chapter on insects is to be deplored because the tradition which has led to the somewhat exclusive attitude of agricultural and horticultural entomologists, though of little importance in practice, has the disadvantage that few 'plant pathologists' are given elementary instruction in entomology as a part of their training.

Two series of chapters on 'Factors influencing the development of pathogenic diseases' and 'Disease control' bring together much scattered information in a manner which clearly shows the late Neil Stevens's stimulating and very personal approach to these problems. Many of the examples used to illustrate the discussion are chosen from diseases which are important in North America, and though for other countries alternative examples would readily suggest themselves, every reader would find it necessary to draw to a greater or less extent on the wealth of data issued by the United States Department of Agriculture. The illustrations are, on the whole, well chosen and the reproductions of a selection of figures from older books and monographs (a feature characteristic of *Chronica Botanica*

publications) give a feeling that the subject has deep roots. The book is well produced and can be warmly recommended to all plant pathologists, and as ancillary reading for any student of the plant sciences. If subsequent volumes of the Manual of the Plant Sciences, 'a comprehensive review of the present state, historical development, and future possibilities of the science of botany', are as useful as this first instalment the series should be welcome.

G. C. AINSWORTH

Anatomy of the Dicotyledons. By C. R. METCALFE and L. CHALK. Pp. 1500. Oxford: Clarendon Press. 1950. 2 volumes. Price £6. 6s. od.

'This reference book aims at providing a summary of our present knowledge of the anatomy of the Dicotyledons.' With this modest statement of their objectives Dr Metcalfe, of the Jodrell Laboratory at Kew, and Dr Chalk, of the Imperial Forestry Institute, open their preface to what will undoubtedly rank as one of the major botanical works of the present century. In the 1500 pages which follow they describe, in note form, the anatomy of the vegetative organs of 263 families of Dicotyledons. For each family there is a general summary of the outstanding anatomical features, followed by more detailed descriptions of leaf, stem, wood and root structure in the different genera. Although primarily a catalogue of anatomical characters there are useful notes for each family on the probable taxonomic and phylogenetic significance of such characters and, for most families, notes on the economic uses. For certain families a short section on ecological anatomy is also included. An attractive and valuable feature of the work are the 317 line drawings, many of them full-page illustrations, which clearly show the range of structure encountered within each family as well as special features of taxonomic value such as hairs, cystoliths, resin ducts, etc.

Although based on Solereder's classic work, *The Systematic Anatomy of the Dicotyledons*, the English translation of which appeared in 1908, this is virtually a new work. It incorporates not only the results from over 2500 new publications on plant anatomy which have appeared since 1908 but also the fruits of much original investigation by the authors themselves. A particularly welcome addition are the detailed descriptions of wood structure prepared by Dr Chalk, which together with the diagrams, drawn at a constant magnification of $\times 50$ throughout, should prove of great value to those concerned with the identification of timbers. Pharmacognosists, too, should find much of interest in this work, though economic botanists generally may regret the lack of any reference to the flowering and fruiting structures of the plant. It is to the taxonomist, however, that the book will make its greatest appeal, for, like Solereder's original text it has been written mainly to emphasize the taxonomic and phylogenetic value of histology.

Plant anatomy, within recent years, has come to be regarded by many botanists as the Cinderella of the botanical sciences, and the publication of this monumental work should do much to restore its former prestige and to direct attention to the fact that comparative anatomy is still an active and progressive field of botanical research.

L. C. LUCKWILL

Principles of Plant Physiology. By JAMES BONNER and ARTHUR W. GALSTON. Pp. 509, with 219 illustrations. San Francisco: W. H. Freeman and Co. 1952. \$5.50.

A just criticism of many otherwise excellent text-books on plant physiology is that they are unbalanced in outlook. Certain aspects of the subject are scarcely mentioned whilst others are dealt with *in extenso*; and, in reading his way through many pages of detailed presentation of facts and discussion of relative merits of rival theories, the student all too often loses sight of the general principles involved.

The present work has been written with the express object of avoiding these errors. The emphasis throughout is on the basic principles, and the authors freely admit that 'detailed discussions of contradictory views have been avoided, as far as possible, in favour of synthesis of existing knowledge into a single working hypothesis'. Whatever one's views on the desirability of this method of presentation, one is bound to agree that this is a most readable book and will do far more to stimulate real interest in plant physiology than many of the more exhaustive text-books on the subject which have appeared within recent years.

It is essentially a work of popular science, using the term in its best sense, and, although intended primarily for students with a background of basic science it will undoubtedly appeal to a much wider public. Those whose interests lie in the applied fields of plant science will find that throughout the book the authors have stressed the close relationship of plant physiology with the practical problems of agriculture and horticulture. The lay reader will find within its pages a most stimulating and lucid summary of modern views on the nutrition, metabolism and growth and development of the flowering plant, whilst the specialist will appreciate the reviews of some of the more recent advances in the subject. On pages 204-13, for instance, is an exceptionally clear and well-illustrated account of the structure of the cell wall as revealed by X-ray and electron microscope studies; and in chapter 19 the problems of differentiation are reviewed in the light of recent knowledge gained from the technique of tissue culture.

The book contains little tabular matter but a very large number of illustrations. Most of these are diagrammatic and quite a few are amusing—or even ludicrous—but all are valuable in helping to build up a visual picture of the principles under discussion. There are few references to original papers, but at the end of each chapter is a list of books and reviews suggested for general reading, which will be of value to students.

L. C. LUCKWILL